PN-ACL-963

1998 MHCA Publications

Investigations supported by the Micronutrient for Health Cooperative Agreement (MHCA)

between the

Center for Human Nutrition,
Department of International Health,
Johns Hopkins School of Hygiene and Public Health

and

Office of Health and Nutrition
US Agency for International Development,
Washington, DC

Preface

Through its agenda of applied research, technical assistance and training the Center for Human Nutrition (CHN), Department of International Health, Johns Hopkins University seeks to significantly advance knowledge and the development and application of methods related to preventing key micronutrient deficiencies that can advance global prevention programs. Much of the CHN's micronutrient research agenda has received support, in whole or in part, by the Office of Health and Nutrition, US Agency for International Development (USAID), through the "Vitamin A for Health" cooperative agreements at Johns Hopkins since the early 1980's, and more recently through its current "Micronutrient for Health" Cooperative Agreement (MHCA).

CHN is committed to the dissemination of major research findings and their relevance to programs and policies through publications in peer-review scientific journals, the lay press, conferences and consultations, and advocacy and constituency-building among legislators and other government bodies. This volume pulls together the publications under the CHN's micronutrient agenda that appeared in peer-review journals from January to December 1998. These studies were supported, in whole or in part, by the "Vitamin A for Health" or "Micronutrient for Health" cooperative agreements with USAID.

B

Table of Contents

1998 Publications *(Peer-reviewed Journal Articles)*

Vitamin A

1. Christian P, West KP Jr, Khatry SK, Katz J, LeClerq SC, Pradhan Ek, Shrestha SR. Vitamin A or B-carotene supplementation reduces but does not eliminate maternal night blindness in Nepal. J Nutr 1998;128:1458-1463.

2. Christian P, West KP Jr., Khatry SK, Katz J, Shrestha SR, Pradhan EK, LeClerq SC, Pokhrel RP. Night blindness of pregnancy in rural Nepal - nutritional and health risks. Int J Epidemiol 1998;27:231-137.

3. Christian P, Schulze K, Stoltzfus RJ, West KP Jr. Hyporetinolemia, illness symptoms, and acute phase protein response in pregnant women with and without night blindness. Am J Clin Nutr 1998;67:1237-1243.

4. Christian P, Bentley ME, Pradhan R, West KP, Jr. An ethnographic study of night blindness 'ratauni' among women in the terai of Nepal. Soc Sci Med 1998;46:879-889.

5. Christian P, Thorne-Lyman AL, West KP Jr., Bentley ME, Khatry SK, Pradhan EK, LeClerq SC, Shrestha SR. Working after the sun goes down: Exploring how night blindness impairs women's work activities in rural Nepal. Eur J Clin Nutr 1998;52:519-524.

6. Gittelsohn J, Shankar AV, West KP Jr., Faroukh F, Gnywali T, Pradhan,E. Child feeding and care behaviors are associated with xerophthalmia in rural Nepalese households. Soc Sci Med 1998;47:477-486.

7. Humphrey JH, Agoestina T, Wu L, Juliana A, Septiana S, Ichord RN, Widjaja H, Cerreto M, Katz J, West KP Jr. Neonatal vitamin A supplementation: effect on development and growth at 3 years of age. Am J Clin Nutr 1998;68:109-117.

8. Khatry SK, Shrestha SR, LeClerq S, Pradhan EK, Christian P, West KP. Maternal night blindness in Nepal: health risks and consequences. J Nepal Med Assoc 1998;37:427-433.

9. Semba RD, Miotti PG, Chiphangwi JD, Yang LP, Saah A, Hoover DR. Maternal vitamin A deficiency and infant mortality in Malawi. J Trop Ped 1998;44:232-234.

10. Semba RD. The role of vitamin A and related retinoids in immune function. Nutr Rev 1998;56:S38-S48.

11. Shankar AV, Gittelsohn J, West KP Jr., Stallings R, Gnywali T, Faruque F. "Eating from a shared plate affects food consumption in vitamin A deficient Nepali children. J Nutr 1998;128:1127-1133.

12. Shankar AV, Gittelsohn J, Pradhan EK, Dhungel C, West KP Jr. Home gardening and access to animals in households with xerophthalmic children in rural Nepal. Food Nutr Bull 1998;19:34-41.

13. Sommer A. Moving from science to public health programs: lessons from vitamin A. Am J Clin Nutr 1998;68:513S-516S.

14. Sommer A. Xerophthalmia and vitamin A status. Progress in Retinal and Eye Research 1998;17:9-31.

15. West KP Jr, LeClerq SC. Vitamin A for health, vision and survival. J Essential Natl Hlth Res Nepal 1998;1:49-50.

Vitamin A and HIV

16. Rwangabwoba JM, Fischman H, Semba RD. Serum vitamin A levels in adults during tuberculosis and human immunodeficiency virus infection. Int J Tuberculosis and Lung Dis 1998;2:771-773.

17. Semba RD, Lyles CM, Margolick J, Caiaffa WT, Farzadegan H, Cohn S, Vlahov D. Vitamin A supplementation and human immunodeficiency virus load in injection drug users. J Infectious Diseases 1998;177:611-616.

18. Semba RD, Miotti PG, Lan Y, Chiphangwi JD, Hoover DR, Dallabetta GA, Yang LP, Saah AJ. Maternal serum lactoferrin and vertical transmission of HIV. AIDS 1998;12:331-332.

Vitamin A and Malaria

19. Lewallen S, Taylor TE, Molyneux ME, Semba RD, Wills BA, Courtright P. Association between measures of vitamin A and the ocular fundus findings in cerebral malaria. Arch Ophthalmol 1998;116:293-296.

Iron Deficiency and Anemia

20. Albonico M, Stoltzfus RJ, Cancrini G, Tielsch JM, Chwaya HM, Ercole E, Savioli L. Epidemiological evidence for a differential effect of hookworm species, A. duodenale or N. americanus, on iron status of children. Int J Epidemiol 1998;27:530-537.

21. Christian P. Antenatal iron supplementation as a child survival strategy. Letter to the editor. Am J Clin Nutr 1998:404-405.

22. Stoltzfus RJ, Albonico M, Chwaya HN, Tielsch, JM, Schulze KJ, Savioli L. Effects of the Zanzibar school-based deworming program on iron status of children. Am J Clin Nutr 1998;68:179-186.

23. Stoltzfus RJ, Chakraborty J, Rice A, de la Briere B, de Francisco A. Plausible evidence of effectiveness of an iron supplementation programme for pregnant and post-partum women in rural Bangladesh. Food Nutr Bull 1998;19:197-204.

24. Zhou LM, Yang W-W, Hua J-Z, Deng C-Q, Tao X, Stoltzfus RJ. Relation of hemoglobin measured at different times in pregnancy to preterm birth and low birth weight in Shanghai, China. Am J Epidemiol 1998;148(10):998-1006 .

Zinc Deficiency

25. Caulfield LE, Zavaleta N, Shankar AH, Merialdi M. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. Am J Clin Nutr 1998;68:499S-508S.

26. Christian PS, West KP Jr. Interactions between zinc and vitamin A: An update. Am J Clin Nutr 1998;68:435S-441S.

27. Shankar AH, Prasad AA. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 1998;68:447S-463S.

Other

28. Caulfield LE, Stoltzfus RJ, Witter FR. Implications of the Institute of Medicine weight gain recommendations for preventing adverse pregnancy outcomes in black and white women. Am J Pub Health 1998;68:499S-508S.

29. Goetghebeur E, Molenberghs G, Katz J. Estimating causal effect of compliance on binary outcome in randomized controlled trials. Statistics in Medicine 1998;17:341-355.

30. Katz J, West KP Jr, LeClerq SC, Thapa MD, Khatry SK, Pradhan EK, Shrestha SR, Pokhrel RP. Agreement between clinical examination and parental morbidity histories for children in Nepal. J Trop Ped 1998;44:225-229.

31. Navitsky RC, Dreyfuss ML, Shrestha J, Khatry SK, Stoltzfus RJ, Albonico M. Ancylostoma duodenale is responsible for hookworm infections among pregnant women in the rural plains of Nepal. J Parasitol 1998;84:647-651.

32. Park SW, Royal W, Semba RD, Wiegand GW, Griffin DE. Expression of adhesion molecules and CD28 on T lymphocytes during human immunodeficiency virus infection. Clinical and Diagnostic Laboratory Immunology 1998;5:583-587.

VITAMIN A

Vitamin A or β-Carotene Supplementation Reduces but Does Not Eliminate Maternal Night Blindness in Nepal[1]

Parul Christian,[2] Keith P. West, Jr., Subarna K. Khatry,* Joanne Katz, Steven LeClerq, Elizabeth Kimbrough Pradhan and Sharada Ram Shrestha*

*Center for Human Nutrition, Department of International Health, School of Hygiene & Public Health, Johns Hopkins University, Baltimore, MD 21205 and *Nepal Intervention Project Sarlahi-NNIPS, The National Society for Eye Health and Blindness Prevention, Nepal Eye Hospital Complex, Tripureswor, Kathmandu, Nepal*

ABSTRACT We investigated the effect of supplementing women weekly with 7000 μg retinol equivalents as preformed vitamin A or β-carotene vs. a placebo, on the incidence of night blindness during pregnancy and the postpartum period in the rural plains of Nepal. Over a period of ~3 y, ~29,000 women of child-bearing age, living in 171 wards that were randomized to one of the three supplements, contributed 9932 first pregnancies. A prospective, weekly surveillance identified night blindness in pregnant women, verified further by detailed questioning about nighttime vision. After delivery, women were also interviewed at ~3 and ~6 mo postpartum to elicit a night blindness history over the preceding 3 mo. Vitamin A supplementation reduced the incidence of night blindness during pregnancy from 10.7% among controls to 6.7% (relative risk 0.62, 95% confidence interval: 0.45–0.85). β-Carotene supplementation had less of an effect (0.83, 0.63–1.11). Among women who took >95% of their vitamin A supplements during pregnancy, incidence of verified night blindness was reduced by 67%. Incidence (per 100 person-years) of night blindness during the first 3 mo postpartum was 11.3 in the control, 4.3 in the vitamin A and 8.7 in the β-carotene groups, yielding corresponding relative risks of 0.38 (0.26–0.55) and 0.77 (0.57–1.04). In the second 3 mo postpartum, both vitamin A and β-carotene reduced night blindness by ~50%. Vitamin A intakes approaching a recommended amount for pregnancy markedly reduced but did not eliminate night blindness in Nepali women. Greater intakes of vitamin A than provided and/or other nutrients may be needed to prevent maternal night blindness in rural South Asia. J. Nutr. 128: 1458–1463, 1998.

KEY WORDS: • *night blindness* • *pregnancy* • *postpartum* • *vitamin A* • *β-carotene* • *Nepal*

Maternal night blindness is receiving increased attention by the nutrition community as evidence accumulates to show that it occurs widely in South and South-East Asia. Studies in Nepal have revealed important facets of this largely ignored and underreported illness. Specifically, recent population surveys report that 10–20% of pregnant women and 6% of lactating women experience night blindness (Demographic Health Surveys 1997, Katz et al. 1995, Linehan et al. 1996). Rural women consider night blindness to be a severe condition of pregnancy and find it deletorious to their daily work activities (Christian et al. 1998a). Night-blind pregnant women are significantly vitamin A–deficient relative to nonnight-blind controls and also face other nutritional and health risks such as wasting malnutrition, anemia and reproductive morbidity (Christian et al. 1998c). In this paper, we examine the efficacy of a weekly, low dose supplement of vitamin A or β-carotene in reducing the incidence of night blindness during pregnancy and the postpartum period in Nepal where such an intervention has demonstrated a reduction in pregnancy-related mortality (West et al. 1997).

MATERIALS AND METHODS

A randomized, double-masked community trial was conducted in Sarlahi District, located in the south-eastern plains of Nepal, from March 1994 to October 1997. Thirty village development communities (VDC)[3] with nine wards in each, participated in the trial. Each week, 7000 μg retinol equivalents (RE) of vitamin A, as either retinyl palmitate or β-carotene (42 mg), or a placebo was given to married women 12–45 y of age (n = ~44,000) to assess the effect on fetal loss, and infant and maternal mortality. Randomization to the three treatment arms was done by ward. From September 1994, a weekly surveillance was set up to identify night-blind pregnant women in 19 of 30 VDC (171 wards with ~29,000 women), allowing us to measure the effect of vitamin A or β-carotene supplementation on the incidence of maternal night blindness. It was hypothesized that

[1] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[2] To whom correspondence should be addressed.

[3] Abbreviations used: CI, confidence interval; RDA, recommended dietary allowance; RE, retinol equivalent; RR, relative risk; VDC, village development communities.

0022-3166/98 $3.00 © 1998 American Society for Nutritional Sciences.

Manuscript received 18 February 1998. Initial review completed 22 March 1998. Revision accepted 26 May 1998.

weekly vitamin A or β-carotene supplementation before and during pregnancy would reduce the occurrence of night blindness during pregnancy and the postpartum period. With a sample size of 3200 per group, there was adequate power (80%) to detect a decrease of >20% in the incidence of night blindness, without any consideration for design effect.

Women contributing pregnancies were interviewed at home within 1–2 wk of reporting their pregnancy, and again, 12 wk later. Trained interviewers obtained information from women on dietary intake, tobacco and alcohol use, and participation in strenous activities in the past 7 d. Data on socioeconomic status was collected at the second interview.

Eliciting night blindness histories. Local female ward distributors, who were responsible for distributing the study supplements, identifying pregnancies and reporting vital events, were trained to elicit a history of night blindness from pregnant women during weekly home visits. The question asked to pregnant women every week was "do you currently have night blindness" using the local terms for the condition in Nepali ("rataundho") and Maithili ("ratauni"), languages spoken in this region. Data from these weekly histories were used to determine the incidence, gestational onset and duration of night blindness during pregnancy. Histories were verified, usually in the following week, by trained interviewers asking about symptoms and the effect night blindness was having on the activity of women. A woman reporting night blindness was considered a "verified case" if she described symptoms of poor or no vision at dusk or night but normal vision during daytime. Night blindness was not treated for the following reasons: *1)* the initial uncertainty of the validity and reliability of its diagnosis based on ~20,000 weekly histories that were obtained by about 300 local women without health training; *2)* insufficient evidence about its extent and associated health risks; and *3)* concerns raised throughout the trial of overdosing with vitamin A pregnant women who were already receiving 7000 μg RE (23,300 IU) as either preformed vitamin A or β-carotene in their respective (masked) treatment arms. A few women who were not reportedly night blind at the time of interview, but reported a recent episode that disappeared either with self-treatment or spontaneously, were also considered "verified." Among women delivering a live birth, histories of night blindness for the previous 3 mo were obtained at the 3- and 6-mo postpartum home visits by trained interviewers. At the 3-mo postpartum interview, cases were also asked about the number of days after delivery when they developed the condition.

Selection criteria. Only women in their first pregnancies enrolled in the trial who had an outcome by the first week of April 1997 (formal closeout for pregnancy outcomes in the trial) were included in the analysis. Also, women with eligible pregnancies had to have met the following criteria: *1)* declared being pregnant for at least 4 wk, *2)* been asked about night blindness for ≥1 wk and *3)* survived the pregnancy. A total of 9932 women over a pregnancy enrollment period of ~3 y were eligible, based on the above criteria, and formed the cohort for the present analysis. Of these, 868 women reported being night blind at least once during their pregnancy.

Statistical analysis. General characteristics of the eligible pregnant women across the three treatment groups were compared by ANOVA and chi-square tests, as appropriate, employing a 5% Type I error to define significance. The incidence rate of night blindness during pregnancy was calculated by dividing the number of pregnant women with a positive history ("cases") by the total number of pregnant women for each treatment group. Relative risks (RR) and 95% confidence intervals (CI) were calculated using the ratio of the incidence rate of night blindness in the vitamin A or β-carotene group to the placebo group (Kahn and Sempos 1989). The confidence intervals were inflated by ~15% (in their natural logarithm) to adjust for clustering of night blindness using the overdispersion from a binomial regression to estimate the design effect (Katz et al. 1988, McCullagh and Nelder 1989). Incidence rates and RR were also calculated stratified by compliance to the supplement. Design effect–adjusted CI were calculated separately for each stratum of compliance.

For the postpartum period, the incidence of night blindness was expressed per 100 person-years to provide a standard period of risk exposure for women who were interviewed at different times for their "3 month" postpartum interview. Only women who developed night blindness in the first 90 d postpartum were included in the calculation of the incidence in the first 3 mo. The incidence rate was stratified by the first, second and third month after delivery to examine change in the risk of night blindness with time since delivery. The second interview was conducted at 6–7 mo postpartum when women were asked if they had developed night blindness since the time they were last interviewed. A small number of women interviewed after 7 mo postpartum were excluded from this analysis to estimate more accurately the incidence of night blindness in the second 3 mo postpartum. The incidence rate was again expressed per 100 person-years to account for the variation in the time of interview in relation to birth. All 95% confidence intervals for postpartum RR were adjusted for the design effect.

The study protocol was reviewed and approved by the Nepal Health Research Council in Kathmandu, Nepal and the Joint Committee on Clinical Investigation at the Johns Hopkins School of Medicine, Baltimore, MD. Verbal informed consent was obtained from women at the time of enrollment in the study and before conducting each interview. Women were free to refuse supplements or interviews at any time during the course of the study.

TABLE 1

General characteristics of pregnant women (at mid-pregnancy) by treatment group in the night blindness study area

	Placebo	Vitamin A	β-Carotene
Pregnant women,[1] *n*	3269	3530	3133
Age,[2]* *y*	23.9 ± 5.2	24.1 ± 5.3	24.3 ± 5.5
MUAC,[2]* *cm*	21.3 ± 1.7	21.4 ± 1.7	21.5 ± 1.7
Socioeconomic status		%	
Literate***	16.9	13.7	18.2
Low caste/non-Hindu***	18.8	26.3	20.9
Landless*	54.7	57.8	56.3
Radios owned, any	29.9	29.1	31.7
Cattle owned, any*	63.9	64.2	66.9
Low quality (thatch/ mud) walls***	87.7	89.9	86.7
Food intake[3] (any in the past wk)			
Meat/fish/eggs*	52.8	50.6	55.2
Dairy products	61.0	59.7	62.1
Yellow fruits/ vegetables***	37.9	34.3	39.7
Dark green leaves*	55.7	57.1	58.9
Activities[3] (past 7 d)			
Carried firewood*	16.6	14.0	16.0
Worked in the fields*	20.2	19.6	22.5
Smoked cigarettes	28.8	27.4	30.1
Drank alcohol***	9.4	7.6	13.2

[1] Data were missing for 187–246 women in each of the three treatment groups.

[2] Values are means ± SD. MUAC, mid-upper arm circumference.

[3] Food intake and activities assessed at pregnancy enrollment.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$ by ANOVA for age and mid-upper arm circumference, otherwise by chi-square.

RESULTS

Pregnant women in the three randomized groups were comparable with regard to demographic, socioeconomic, nutritional and other characteristics (**Table 1**). Slightly more women in the vitamin A group were poorer than in the other two groups. Most differences were small and were significant because of the large sample size. Fifty to sixty percent of women had eaten any preformed sources of vitamin A or dark green leaves, but only 30–40% had eaten any yellow fruits and vegetables in the previous week. Strenuous work (e.g., working in

6

TABLE 2

Effect of maternal vitamin A and β-carotene supplementation on the incidence of night blindness during pregnancy

	Placebo	Vitamin A	β-Carotene
Cases, *n*	351	236	281
Pregnant women, *n*	3269	3530	3133
Incidence rate, %	10.7	6.7	8.9
RR[1] (95% CI)	1.0	0.62 (0.45–0.85)	0.84 (0.63–1.11)
Interviewed,[2] *n*	332	205	252
Verified as night blind, *n*	278	147	195
Incidence rate, %	8.6	4.2	6.3
RR (95% CI)	1.0	0.49 (0.33–0.72)	0.73 (0.52–1.03)

[1] RR, relative risk; CI, confidence interval.
[2] Histories of women reporting night blindness were verified by a trained interviewer of nighttime and daytime symptoms of vision problems. Data on 19, 31 and 29 women in the placebo, vitamin A and β-carotene groups, respectively, are missing.

TABLE 3

Incidence of verified cases of night blindness in pregnant women by compliance to vitamin A and placebo supplements during pregnancy

	Incidence rate (%)			
Compliance[1] (%)	Placebo	Vitamin A	RR[2]	95% CI[2]
96–100 (4th quartile)	9.6	3.2	0.33	(0.18–0.59)
86–95 (3rd quartile)	11.8	4.5	0.38	(0.22–0.64)
66–85 (2nd quartile)	9.3	4.5	0.48	(0.28–0.82)
40–65 (10–25th percentile)	6.6	3.7	0.57	(0.29–1.14)
<40 (<10th percentile)	4.0	4.8	1.2	(0.67–2.14)

[1] Compliance was defined as the percentage of all weekly supplements consumed by women from ~1 m before conception until the end of pregnancy.
[2] RR, relative risk; CI, confidence interval (corrected for design effect).

the fields or carrying firewood) was reported by 15–20% of women. Almost 30% of the women had smoked cigarettes and 8–13% had drunk alcohol in the previous week.

The incidence of night blindness during pregnancy was 10.7% in the group receiving placebo vs. 6.7% in the vitamin A and 8.9% in the β-carotene groups (Table 2). This yielded RR (95% CI) of 0.62 (0.45–0.85) and 0.84 (0.63–1.11), or 38 and 16% reductions in night blindness with vitamin A and β-carotene supplementation, respectively. Interviewer-verified rates of night blindness were slightly lower but associated with stronger protective effects, with RR (95% CI) of 0.49 (0.33–0.72) in the vitamin A group and 0.73 (0.52–1.03) in the β-carotene group. This suggests that misclassification of night blindness from histories elicited by local supplement distributors weakened the estimated efficacy of vitamin A or β-carotene by 11–13% in this population. The higher effect of vitamin A vs. β-carotene supplementation was not significant for either unverified (RR = 0.74, 95% CI = 0.63–0.88) or verified night blindness (RR = 0.62, 95% CI = 0.62–0.86).

Varying compliance to the supplement regimen altered the effect of vitamin A in reducing night blindness during pregnancy (Fig. 1). Compliance was defined as the proportion of weekly doses a woman received from ~1 mo before conception until the end of pregnancy. The percentage of compliance was categorized into five groups of decreasing levels. The first three categories represented the upper three quartiles of the distribution. The last two categories divided the lowest quartile into the 10–25th percentiles and <10th percentile of the distribution. Incidence of night blindness in the placebo group was much higher than in the vitamin A group among women consuming 86–100% of their supplements (3rd and 4th quartiles). The RR associated with vitamin A receipt in these groups was 0.4–0.5, indicating a 50–60% reduction in night blindness. The effect was lowered to ~40% reduction in the compliance range of 66–85%. At ≤65% compliance, there was no apparent protection from night blindness with vitamin A supplementation as shown by a RR of ~1.0. A similar analysis of β-carotene resulted in a small increase in the protective effect of β-carotene among high (96–100%) compliers, but the 95% confidence interval about the RR did not exclude 1.0.

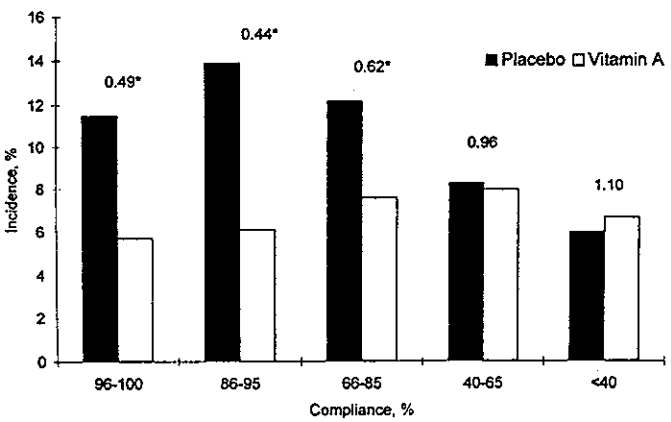


FIGURE 1 Incidence of night blindness in pregnant women by level of compliance to vitamin A and placebo supplements during pregnancy. Relative risks of night blindness in the vitamin A group compared with the placebo group for each compliance stratum are given above the bars; *95% confidence interval (corrected for design effect) excludes 1.0.

We further examined the incidence of verified night blindness by compliance to vitamin A supplementation during pregnancy (Table 3). There was a strong dose-responsive relationship between compliance to the supplement and relative risk of developing night blindness during pregnancy. Among women taking 96–100% of their supplements, vitamin A reduced the risk of night blindness by 67% (RR = 0.33). This estimate, among highest compliers, approximates the efficacy of this level and frequency of vitamin A supplementation in preventing night blindness in pregnancy. Vitamin A supplementation did not reduce the occurrence of interviewer-verified night blindness at compliance levels <40%. In the case of β-carotene, verification did not appear to improve its efficacy; the RR estimated among those with 96–100% was 0.72, almost identical to the RR estimate of 0.73 observed on average.

Onset of night blindness occurred, on average, at 27 wk gestation in the placebo group, compared with a slightly earlier onset in the vitamin A (24 wk) and β-carotene groups (26 wk) (Table 4). Episodes also lasted longer in the placebo (5.0 wk) than vitamin A (3.7 wk) and β-carotene groups (4.4 wk).

The incidence rate (per 100 person-years) of night blindness during the first 3 mo postpartum was reduced from 11.3 in the placebo group to 4.3 in the vitamin A and 8.7 in the β-carotene groups, yielding corresponding RR (95% CI) of 0.38 (0.26–0.55) and 0.77 (0.57–1.04) (Table 5). The risk of night blindness was the highest in the first 30 d after delivery

7

TABLE 4

Onset and duration of night blindness during pregnancy in women according to treatment group

	Placebo	Vitamin A	β-Carotene
Onset, *week of gestation*	26.8 ± 8.5	24.2* ± 8.7	25.8 ± 8.6
Duration, *wk*	5.0 ± 6.3	3.7* ± 4.9	4.4 ± 5.4

[1] Values are means ± SD, n = 351 in placebo, 236 in vitamin A and 281 in the β-carotene groups.
* Significantly ($P < 0.001$) different from the placebo group.

(Fig. 2). It was halved in mo 2 and dropped further in mo 3 postpartum. Vitamin A supplementation significantly reduced the risk of night blindness during all three time periods. The early 1-mo specific risk of night blindness in the β-carotene group was lower than that of the placebo group, but this difference was not significant. The incidence rate (per 100 person-years) in the second 3-mo postpartum was lower, i.e., 7.1 in the placebo group, 3.6 in the vitamin A group and 3.2 in the β-carotene group. Both vitamin A and β-carotene supplementation reduced night blindness in the second 3 mo by ~50%.

DISCUSSION

The occurrence of night blindness during pregnancy was high in this Nepali population as indicated by an incidence rate of 11 cases per 100 pregnancies for groups without intervention. Because women were often not observed for their entire pregnancy, the observed incidence rates may underestimate actual rates during pregnancy. These data reveal, as previously reported (Katz et al. 1995), that maternal night blindness is a problem of public health significance in the rural plains of Nepal. Serum retinol concentrations available for 117 verified night-blind women and their 117 matched controls in groups without intervention were strongly associated with night blindness (Christian et al. 1998c), providing evidence of the validity of maternal night blindness histories in a subsample of women.

About 60% of the risk of night blindness during pregnancy remained unaltered with vitamin A supplementation. This was unexpected because night blindness among children has been shown to be virtually eliminated with high dose vitamin A supplementation (Sinha and Bang 1976, Sommer and West 1996). We used a weekly dosage that provided slightly more than 1 recommended dietary allowance (RDA) (for periods of pregnancy and lactation) of vitamin A (7000 μg RE). This dosage may not have been adequate to prevent night blindness during pregnancy even for 33% of interviewer-verified cases among the highest compliers. The current WHO recommendation for treating women of reproductive age with night blindness or Bitot's spots is 1501–3003 μg RE of vitamin A/d or 7507 μg RE/wk for at least 4 wk (WHO 1997), although to our knowledge, there have been no trials demonstrating the efficacy of these regimens in doing so. The latter weekly recommendation is similar to the prophylactic dosage used in this study. Although our findings do not relate to treatment, they suggest a need to re-evaluate the treatment recommendation and possibly revise it upward to maximize efficacy and to prevent relapse among vitamin A–responsive cases.

TABLE 5

Effect of vitamin A and β-carotene supplementation on night blindness during the postpartum period

Period postpartum	Placebo	Vitamin A	β-Carotene
0–3 mo			
Cases, *n*	87	35	64
Person-years of follow-up	769	816	735
Incidence, *100 person-years*	11.3	4.3	8.7
RR[1] (95% CI)	1.0	0.38 (0.26–0.55)	0.77 (0.57–1.04)
3–6 mo			
Cases, *n*	70	40	30
Person-years of follow-up	983	1098	932
Incidence, *100 person-years*	7.1	3.6	3.2
RR (95% CI)	1.0	0.51 (0.29–0.81)	0.45 (0.24–0.83)

[1] RR, relative risk; CI, confidence interval.

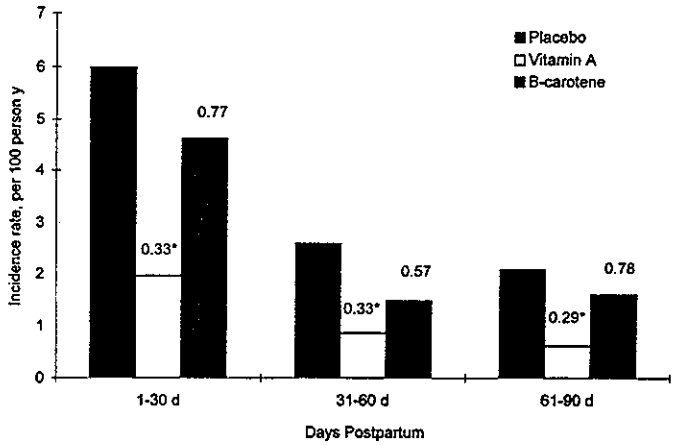


FIGURE 2 Incidence of night blindness by number of days postpartum in women receiving placebo, vitamin A and β-carotene supplements. Relative risks of night blindness in the vitamin A and β-carotene groups compared with the placebo group for each stratum of postpartum days are given above the bars; *95% confidence interval (corrected for design effect) excludes 1.0.

The additional requirement for vitamin A in a healthy pregnancy is relatively low, and confined mostly to the third trimester (Underwood 1994). The late onset of night blindness in pregnancy suggests that fetal demands on maternal vitamin A stores late in gestation may exacerbate a chronic, low vitamin A status in women. Findings of a previous case-control study in this population showed a dramatic difference in the serum retinol level of women with and without night blindness during pregnancy (0.72 vs. 1.03 μmol/L). Fifty-two percent of cases vs. 21% of controls had a serum retinol concentration <0.7 μmol/L (Christian et al. 1998c). High levels of infection may also contribute to an increased demand for vitamin A and a consequent lowering of vitamin A status during pregnancy. In Nepal, night blind women were at a 14-fold higher risk of diarrhea in the past week (Christian et al. 1998b). An acute phase protein response in pregnant women with diarrhea was associated with a 0.54 μmol/L decrease in the serum retinol level, suggesting that infection-induced hyporetinolemia may further predispose women to night blindness during pregnancy, especially in those already vitamin A deplete (Christian et al. 1998b).

Other etiologies such as zinc deficiency, which may limit vitamin A utilization (Christian and West 1998, Smith 1980) and is believed to be widespread (Caulfield et al. 1998), may

contribute to night blindness of pregnancy. In the retina, retinol is converted to retinal, the form required to synthesize the photosensitive pigment rhodopsin, through a reaction requiring a zinc-dependent retinol dehydrogenase (Huber and Gershoff 1975). Zinc deficiency could inhibit this reaction and result in abnormal dark adaptation or night blindness. Zinc is also required for retinol-binding protein synthesis; thus, its deficiency could impair vitamin A transport (Duncan and Hurley 1978, Mejia 1986). In pregnancy, maternal plasma zinc starts to decrease as early as 6 wk of gestation and continues to decline until delivery (Tamura and Goldberg 1996). Fetal demands on zinc appear to accelerate, especially in the third trimester of pregnancy (Reifen and Zlotkin 1993), which could in part underlie both an increased risk of night blindness at this stage of gestation as well as its partial nonresponsiveness to vitamin A, as seen in this study. Riboflavin deficiency has also been associated with night blindness in adults (Venkataswamy 1967). These studies suggest that multiple nutrient supplementation or a more varied diet may be required to prevent maternal night blindness.

In this trial, β-carotene supplementation, given at presumed retinol equivalency, was not as efficacious as vitamin A in either reducing night blindness or improving circulating retinol levels during pregnancy (West et al. 1997). This may have been because β-carotene did not efficiently convert to vitamin A in this undernourished population. Factors such as intestinal helminths, protein-energy malnutrition and deficiencies of zinc, iron and vitamin A could influence the conversion of β-carotene to retinol (Erdman et al. 1993). β-Carotene may also be redirected for other immunoregulatory (Prabhala et al. 1991) and antioxidant functions (Allard et al. 1994) that are independent of its role as provitamin A.

Compared with the pregnancy period, the efficacy of both low dose vitamin A and β-carotene appeared to be higher during the postpartum period. Although the requirements of vitamin A are greater in lactation than in pregnancy (NRC 1989), the risk of night blindness in breastfeeding populations has been reported to be higher during pregnancy (Katz et al. 1995, Malyavin et al. 1996). Factors such as plasma volume expansion of pregnancy (Kelner et al. 1969, McGanity et al. 1954), which can lower maternal plasma retinol over the course of the pregnancy, and favored partitioning of vitamin A toward fetal tissues in women with marginal or low serum retinol levels (Butte and Calloway 1982, Gebre-Medhin and Vahlquist 1984, Shirali et al. 1989) may make women more vulnerable to vitamin A deficiency during late pregnancy than lactation. Pregnancy may also be a unique state during which other etiologies such as zinc deficiency could influence dark adaptation. Local cultural practices promoting better diets in the postpartum period relative to the pregnancy period could also explain a lower incidence of night blindness during lactation.

The risk of night blindness after delivery was highest in the first month, declining thereafter. This suggests that the nutritional demands of recovering from birth and establishing lactation, especially given high maternal losses of vitamin A in colostrum and immature breastmilk (Patton et al. 1990) may be high in the first month. In the second 3 mo of lactation, the incidence of night blindness was lower, and vitamin A and β-carotene supplements had a similar relative effect on its occurrence. Several countries, including Nepal, have recently adopted a policy to dose women with 60,060 μg RE of vitamin A within the first 6 wk of delivery to improve the health and status of infants. Although this policy remains to be implemented nationally in Nepal, it may have a potential benefit on preventing the maternal night blindness that occurs in the first few months after birth.

An adequate dietary or supplemental intake of vitamin A throughout pregnancy and postpartum periods is necessary but not sufficient for eliminating maternal night blindness. For a substantial proportion of women, who are exposed to repeated pregnancies in poor, chronically malnourished societies, a recommended dietary allowance may be insufficient to maintain adequate maternal vitamin A status and prevent occurrence of night blindness during pregnancy and lactational postpartum periods. It is also possible that other nutritional deficiencies, such as that of zinc, may limit the efficacy of vitamin A in preventing maternal night blindness, serving to reinforce the need for a balanced diet to maintain adequate health and function of women during and after pregnancy.

ACKNOWLEDGMENTS

This study was done in collaboration between the Center for Human Nutrition, Department of International Health at the Johns Hopkins School of Public Health and the National Society for Eye Health and Blindness Prevention (Nepal Netra Jyoti Sangh), Kathmandu, Nepal, supported under Cooperative Agreement DAN 0045-A-00-5094-00 between the Johns Hopkins University, Baltimore, MD and the office of Health and Nutrition, U.S. Agency for International Development (USAID), assisted by Task Force Sight and Life, Basel, Switzerland and the Sushil Kedia Foundation, Hariaun, Sarlahi, Nepal. We thank members of the Nepal Nutrition Intervention Project-Sarlahi in Nepal (beyond the authors) for their assistance in the conduct of this study, especially R. Adhikari, S. M. Dali, R. P. Pokhrel and R. J. Stoltzfus; D. N. Mandal, T. R. Shakiya, G. Subedi, U. Shankar, A. Bhetwal and D. B. Khadka.

LITERATURE CITED

Allard, J. P., Royall, D., Kurian, R., Muggli, R. & Jeejeebhoy, K. N. (1994) Effects of beta-carotene supplementation on lipid peroxidation in humans. Am. J. Clin. Nutr. 59: 884–890.

Butte, N. F. & Calloway, D. H. (1982) Proteins, vitamin A, carotene, folacin, ferritin and zinc in Navajo maternal and cord blood. Biol. Neonate 41: 273–278.

Caulfield, L. E., Zavaleta, N., Shankar, A. H. & Merialdi, M. (1998) The potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. Am. J. Clin. Nutr. (in press).

Christian, P., Bentley, M. E., Pradhan, R. & West, K. P., Jr. (1998a) An ethnographic study of night blindness (ratauni) among women in the terai of Nepal. Soc. Sci. Med. 46: 879–889.

Christian, P., Schulze, K., Stoltzfus, R. J. & West, K. P., Jr. (1998b) Hyporetinemia, illness symptoms, and acute phase protein response in pregnant women with and without night blindness. Am. J. Clin. Nutr. 67: 1237–1243.

Christian, P. & West, K. P., Jr. (1998) Interactions of zinc and vitamin A. An Update. Am. J. Clin. Nutr. (in press).

Christian, P., West, K. P., Jr., Khatry, S. K., Katz, J., Shrestha, S. R., Pradhan, E. K., LeClerq, S. C. & Pokhrel, R. P. (1998c) Night blindness of pregnancy in rural Nepal—nutritional and health risks. Int. J. Epidemiol. 27: 231–237.

Demographic and Health Surveys (1997) Nepal Family Health Survey 1996. Macro International Inc., Calverton, MD.

Duncan, J. R. & Hurley, L. S. (1978) An interaction between zinc and vitamin A in pregnant and fetal rats. J. Nutr. 108: 1431–1438.

Erdman, J. W., Jr., Bierer, T. L. & Gugger, E. T. (1993) Absorption and transport of carotenoids. In: Carotenoids in Human Health (Canfield, L. M., Krinsky, N. I. & Olson, J. A., eds.), pp. 76–85. The New York Academy of Sciences, New York, NY.

Gebre-Medhin, M. & Vahlquist, A. (1984) Vitamin A nutrition in the human fetus. Acta Pediatr. Scand. 73: 333–340.

Huber, A. M. & Gershoff, S. N. (1975) Effects of zinc deficiency on the oxidation of retinol and ethanol in rats. J. Nutr. 105: 1486–1490.

Kahn, H. A. & Sempos, C. T. (1989) Statistical Methods in Epidemiology, pp. 45–71. Oxford University Press, New York, NY.

Katz, J., Khatry, S. K., West, K. P., Jr., Humphrey, J., LeClerq, S. C., Kimbrough-Pradhan, E., Pokhrel, R. P. & Sommer, A. (1995) Night blindness is prevalent during pregnancy and lactation in rural Nepal. J. Nutr. 125: 2122–2127.

Katz, J., Zeger, S. L. & Tielsch, J. M. (1988) Village and household clustering of xerophthalmia and trachoma. Int. J. Epidemiol. 17: 865–869.

Kelner, M., Gomes, S.M.F., Madruga, I., Valenca, T., Abath, E. C. & Linhares, E. (1969) Vitamin A e caroteno no ciclo gravido-puerperal. Rev. Bras. Med. 26: 520–531.

Linehan, M., Shrestha, A., Ban, B. & Rycus, P. (1996) Xerophthalmia preva-

9

lence in ten districts of the far and mid-western regions of Nepal. XVI International Vitamin A Consultative Group meeting. Guatemala City, Guatemala (abs.).

Malyavin, A., Beauphanny, V., Arouny, A. & Cohen, N. (1996) National vitamin A survey in Lao PDR. XVI International Vitamin A Consultative Group meeting. Guatemala City, Guatemala (abs.).

McCuliagh, P. & Nelder, J. A. (1989) Generalized Linear Models, 2nd ed. Chapman and Hall, New York, NY.

McGanity, W. J., Cannon, R. O., Bridgforth, E. B., Martin, M. P., Densen, P. M., Newbill, J. A., McClennan, G. S., Christie, A., Peterson, J. C. & Darby, W. J. (1954) The Vanderbilt cooperative study of maternal and infant nutrition. IV. Relationship of obstetric performance to nutrition. Am. J. Obstet. Gynecol. 67: 501–527.

Mejia, L. A. (1986) Vitamin A-nutrient interrelationships. In: Nutrition Basics and Applied Science—Q series of monographs (Bauernfeind J. C., ed.), pp. 69–100. Academic Press, Inc., New York, NY.

National Research Council (1989) Recommended Dietary Allowances, 10th ed. National Academy Press, Washington, DC.

Patton, S., Canfield, L. M., Huston, G. E., Ferris, A. M. & Jensen, R. G. (1990) Carotenoids of human colostrum. Lipids 25: 159–165.

Prabhala, R. H., Garewal, H. S., Hicks, M. J., Sampliner, R. E. & Watson, R. R. (1991) The effects of 13-*cis*-reinoic acid and beta-carotene on cellular immunity in humans. Cancer 67: 1556–1560.

Reifen, R. M. & Zlotkin, S. (1993) Microminerals. In: Nutrition Needs of the Preterm Infant. Scientific Basis and Practical Guidelines (Tsang, R. C., Lucas, A., Uauy, R. & Zlotkin, S., eds.), pp. 195–207. Williams and Wilkins, Baltimore, MD.

Shirali, G. S., Oelbr, D. G. & Mehta, K. P. (1989) Maternal-neonatal serum vitamin A concentrations. J. Pediatr. Gastroenterol. Nutr. 9: 62–66.

Sinha, D. P. & Bang, F. B. (1976) The effect of massive doses of vitamin A on the signs of vitamin A deficiency in preschool children. Am. J. Clin. Nutr. 29: 110–115.

Smith, J. C. (1980) The vitamin A zinc connection: a review. Ann. N.Y. Acad. Sci. 355: 62–75.

Sommer, A. & West, K. P., Jr. (1996) Vitamin A Deficiency: Health, Survival, and Vision. Oxford University Press, New York, NY.

Tamura, T. & Goldenberg, R. L. (1996) Zinc nutriture and pregnancy outcome. Nutr. Res. 16: 139–181.

Underwood, B. A. (1994) Maternal vitamin A status and its importance in infancy and early childhood. Am. J. Clin. Nutr. 59 (suppl.): 517S–524S.

Venkataswamy, G. (1967) Ocular manifestation of vitamin B-complex deficiency. Br. J. Ophthalmol. 51: 749–754.

West, K. P., Jr., Khatry, S. K., Katz, J., LeClerq, S. C., Pradhan, E. K., Shrestha, S. R., Connor, P. B., Dali, S., Adhikari, A. & Pokhrel, R. P. (1997) Impact of weekly supplementation of women with vitamin A or beta-carotene on fetal, infant, and maternal mortality in Nepal. XVIII International Vitamin A Consultative Group Meeting, Cairo, Egypt.

World Health Organization (1997) Vitamin A supplements. A guide to their use in the treatment and prevention of vitamin A deficiency and xerophthalmia, 2nd ed. WHO, Geneva, Switzerland.

10

© International Epidemiological Association 1998 Printed in Great Britain
International Journal of Epidemiology 1998;27:231–237

Night blindness of pregnancy in rural Nepal—nutritional and health risks

Parul Christian, Keith P West Jr, Subarna K Khatry, Joanne Katz, Sharda Ram Shrestha, Elizabeth K Pradhan, Steven C LeClerq and Ram P Pokhrel

Background Night blindness (XN) is the most common clinical symptom of vitamin A deficiency among children in developing countries. Yet little is known about the aetiology or associated risks of maternal XN. Emerging evidence from South East Asia suggests that it may be more frequent than previously thought in women of reproductive age, especially during pregnancy.

Methods A population-based, case-control study was conducted to reveal the epidemiology of XN among pregnant Nepali women. Night blind cases were identified by history through a weekly community surveillance system. Controls were randomly selected from a pool of pregnant women without XN and pair-matched for gestational age of the cases. A home-based assessment was done within a week of selection, at which 7-day food frequency and morbidity histories were collected, anthropometry measured, and capillary blood drawn for serum retinol, β-carotene and haemoglobin (Hb) estimation.

Results Cases and controls did not differ by age or number of previous pregnancies. However, cases were more likely to be from the lower castes, be illiterate, live in poorer quality homes, and own no land. The mean serum retinol level of cases was ~0.30 μmol/l lower than controls ($P < 0.001$), indicating a low vitamin A status of night blind pregnant women. Mean Hb level was significantly lower (by 0.7 g/dl, $P < 0.004$), and the risk of severe anaemia (Hb <7.0 g/dl) higher among cases than controls (odds ratio = 3.0, 95% CI : 1.25–7.23). Cases were more undernourished than controls reflected by lower mean weight (–2.6 kg), body mass index (–0.8), arm circumference (–0.9 cm) and triceps skinfold (–0.8 mm). Night blindness was associated with less frequent consumption of preformed vitamin A (milk products, fish and meat) and provitamin A (dark green leafy vegetables and mangoes) foods, especially in summer. Night blind women were 2–3 times more likely to report symptoms of urinary/reproductive tract infections such as lower abdominal pain, painful and burning urination, or vaginal discharge, symptoms of diarrhoea/dysentery, of pre-eclampsia or eclampsia, and of nausea, vomiting or poor appetite throughout pregnancy than controls.

Conclusion Women who experience XN during pregnancy have a low vitamin A status, although several other risk factors appear to cluster among these women as well. Night blind women are also more likely to be anaemic, ill, and acutely undernourished, and to be consuming a nutritionally poorer diet in pregnancy than non-night blind pregnant women. A simple history of XN can identify women at high risk during pregnancy who may require special nutritional support, antenatal care and counselling.

Keywords Night blindness, pregnancy, vitamin A deficiency, Nepal

Accepted 7 July 1997

Center for Human Nutrition, and the Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD, USA and National Society for the Prevention of Blindness (Nepal Netra Jyoti Sangh), Kathmandu, Nepal.

Reprint requests to: Parul Christian, Division of Human Nutrition, Room 2505, Johns Hopkins School of Hygiene and Public Health, 615 N Wolfe St, Baltimore, MD 21205, USA.

11

Maternal night blindness (XN) has received little attention as a problem of potential public health significance in developing countries. Early accounts, found in two clinic-based studies in the late 1960s from India, reported that XN occurred frequently among pregnant Indian women, most often during the third trimester followed by spontaneous remission soon after parturition.[1, 2] Some amelioration following treatment with high doses of vitamin A was observed among pregnant women who presented at the clinic with XN. Recent studies suggest XN to be widespread, affecting 10–50% of women during pregnancy in rural South East Asia.[3–6] Night blindness in these studies was ascertained by obtaining a simple history from the women, either concurrently or retrospectively. Although maternal XN is believed to be a manifestation of vitamin A deficiency, as it is in young children, none of these studies have examined this association. In the south-eastern plains of Nepal, XN affects 10–20% of women during pregnancy, tends to recur with repeated pregnancies[3] and is recognized as an important health problem among women.[7] Night blindness in pregnancy, thus, exists among South East Asian women but its aetiology or associated risk factors, severity, consequences, and public health significance have not been elucidated.

We report findings of a population based case-control study that examined nutritional and other risk factors associated with XN during pregnancy in rural Nepal.

Methods

Study area and population

The study was conducted in Sarlahi District, in the low-lying, south-eastern plains of Nepal called the 'terai'. The altitude of the terai is approximately 250 feet above mean sea level. The climate is hot and wet during the summer monsoon (July–September), cool and dry during winter (October–March) and hot and dry from April to June. A beneficial impact of vitamin A supplementation on child mortality has been well established in this area,[8, 9] where xerophthalmia affects 2–3% of preschool children.[10–12] A randomized community trial, to assess the benefit of weekly, maternal, low-dose, vitamin A and β-carotene supplementation on fetal and early infant survival and growth is currently underway in 30 village development committees (VDC) of this district covering a total population of ~165 000 people. Supplements are delivered by local female workers who also identify pregnancies. Women who report being pregnant are enrolled for an initial in-depth interview about diet, morbidity history, and other risk factors in the previous week.

Surveillance of night blindness

A surveillance system for identifying night blind pregnant women was set up in August 1994 as part of the pregnancy surveillance for the above trial in 19 of the 30 study VDC that were accessible by road. A weekly history of XN was obtained by the female workers from the time women reported being pregnant until the termination of pregnancy. Women were asked: 'Are you currently suffering from night blindness?' using local terms for night blindness (*ratauni* in Maithili and *rataundho* in Nepali). Day blindness in women reporting XN was verified by asking them if they could see clearly during the daylight hours. Those reporting XN who had poor or no vision in the evening or at night, but who had no difficulty seeing during the day were defined as night blind cases and were eligible for enrolment in the study.

Study design

A population-based, pair-matched, case-control design was used to examine risk factors associated with XN. Cases and controls (n = 117 pairs), for the present analysis, were restricted to the cohort of pregnant women derived from the placebo arm of those enrolled in the maternal supplementation trial. After the initial five prevalent cases with pre-existing XN identified in the first week of surveillance, the remaining were all incident cases. For each verified case, a control was randomly selected from a list of non-night blind pregnant women from the 19 VDC and matched with the case with respect to months pregnant at the time of enrolment. Three women initially assigned control status, but who subsequently developed XN in pregnancy, were redesignated as cases, randomly assigned a matched control, and interviewed and examined a second time as a case.

After selection, cases and controls were visited in their homes within a week for an interview and examination. Data were collected on women's previous 7-day frequency of intake of certain vitamin A foods and morbidity history, breastfeeding practice, previous history of XN, and a number of household characteristics and socioeconomic status (SES). Capillary blood was drawn by finger stick for serum retinol, β-carotene, and haemoglobin (Hb) estimation. Anthropometric assessment included weight, height, mid upper arm circumference (MUAC), and triceps skinfold (TSF), using the methodology described by Gibson.[13] Arm muscle and fat area were calculated using the formulas given by Gibson.[13] Wasting status was assessed by calculation of the body mass index (BMI) ($wt\ [kg]/ht^2[m]$). Blood was collected in microtainers and stored in ice in the field, centrifuged within 4–5 h of collection, and the serum obtained stored and transported in liquid nitrogen to the Center for Human Nutrition Laboratory of the Johns Hopkins University in Baltimore, USA. Once in Baltimore, serum samples were stored in a −70°C freezer until the time of analysis. Retinol and β-carotene were analysed by the reversed phase HPLC method. Haemoglobin concentration was measured using a portable, photometric haemoglobinometer (HemoCue Inc., Mission Viejo, California, USA). Severe anaemia in women was treated with 30 days supplement of iron-folate. Serum retinol and β-carotene values were available for 108 pairs; missing values resulted either due to refusal to give blood or insufficient quantities of serum collected and this occurred randomly in cases and controls.

Statistical analysis

All analyses were performed in SAS version 6.1 (SAS Institute Inc., Cary, North Carolina, USA). Frequency distributions, stem and leaf plots, histograms and scatter plots were used to examine the central tendencies, ranges and distributions of all continuous variables such as age, serum retinol levels, anthropometric measurements and Hb levels. Frequency distributions of categorical and ordinal variables were also examined. This was an aid in deciding appropriate cutoffs to use for presenting descriptive data. Contingency tables were prepared in order to make comparisons between cases and controls by SES factors, weekly food intake, weekly morbidity history and other risk factors. To examine univariate differences in dichotomous variables between

cases and controls, McNemar's odds ratios (OR) using the ratio of discordant pairs and test based, 95% confidence intervals (CI) were calculated.[14] The paired t-test was used to compare the mean differences in continuous variables between cases and controls.

Multivariate conditional logistic regression analysis that calculated maximum likelihood estimates[14] was done to examine the associations between risk factors and case-control status. Case status was the dependent variable, while main effects examined were vitamin A food intake, serum retinol, anthropometric and Hb measurements, and past 7-day morbidity history, controlling for the confounding effects of some variables like MUAC, and SES. The OR estimates were calculated from the logistic regression parameter estimates and 95% CI from their standard errors by published methods.[14] The independent variables were entered into the models as dichotomous or indicator variables when they were categorical.

Guttman scaling of socioeconomic status

Guttman scaling, also known as scalogram analysis or cumulative scaling, was used to develop an SES scale using household SES characteristics.[15] This procedure is designed to order both items and subjects with respect to some underlying cumulative dimension. Guttman analysis was done with the SAS version 5.1 (SAS Inc.) PROC Guttman procedure using various combinations of dichotomized SES variables. After several iterations of this procedure, using various possible combinations of SES items, the combination of variables that produced the best scale was ownership of (a) land (b) cattle (c) upper storey to the house and (d) cart. A five-point scale that ranged from 0 to 4, was computed by summing the four SES dichotomous variables. This scale was further dichotomized into <2 versus ≥2 in order to classify families into low and high SES groups. This dichotomous variable correlated well with other indicators such as literacy and caste and was used in the multivariate analysis to control for SES.

Ethical approval

Approval to conduct the study was obtained from the Nepal Health Research Council, Kathmandu Nepal, and the Committee on Human Research at the Johns Hopkins School of Hygiene and Public Health, in Baltimore, MD, USA. Verbal consent was obtained from each subject before enrolling them in the study.

Results

Cases and controls were similar with respect to age and the number of previous pregnancies but varied by SES (Table 1). Night blind women were less likely to be literate than controls, (OR = 0.47, not statistically significant), belonged to the low Hindu-Vaishya or Shudra castes (OR = 4.0), were less likely to own large plots of land (OR = 0.31), have an upper storey to their house (OR = 0.44), and were twice as likely to have homes made from mud and thatched walls or roofs than their controls. Using the Guttman scale case households were half as likely as control households to be of higher SES.

The mean gestational age of both cases and controls at the time of the home-based assessment was 7 months. Cases were 4.5 times more likely (95% CI : 2.2–9.0) to report XN in a previous pregnancy than controls. Both groups were equally likely to breastfeed during pregnancy; about 70% of the multiparous women had breastfed their previous child into the current pregnancy.

Table 1 Demographic and socioeconomic characteristics of cases and controls

	Case (%)	Control (%)	Matched odds ratio	95% CI
Age (years)				
<20	13.8	18.8	1.0	–
20–29	68.9	65.8	1.56	(0.68–3.57)
≥30	17.2	15.4	1.33	(0.3–5.93)
Previous pregnancies				
≤2	55.2	53.0	1.0	–
>2	44.8	47.1	1.08	(0.62–1.89)
Literate	5.9	12.6	0.47	(0.19–1.12)
Caste[a]				
Hindu-High	11.9	26.8	1.0	–
Hindu-Low	69.3	50.5	4.0[b]	(1.62–9.89)
Other	18.8	22.7	1.50	(0.25–8.87)
Land				
1–9 kattha[c]	34.8	32.9	0.88	(0.32–2.41)
≥10 kattha	30.3	42.8	0.31[b]	(0.15–0.65)
Thatch/mud wall	68.1	45.3	2.47[b]	(1.48–4.14)
Thatch roofed	41.8	30.2	1.83[b]	(1.04–3.23)
Upper floor	32.5	49.6	0.44[b]	(0.24–0.80)
High SES scale[d]	56.4	73.9	0.47[b]	(0.27–0.82)

[a] High: Brahmin or Chetri, Low: Vaishya or Shudra, Other: Muslim and Buddhist.

[b] Identifies odds ratios with 95% CI that exclude 1.0.

[c] One kattha = 0.034 hectare or 0.084 acre.

[d] Dichotomized Guttman scale (<2 versus ≥2) ranging from 0 to 4 created using ownership of land, cattle, upper floor and cart.

The mean serum retinol level of cases was significantly lower (by ~0.30 μmol/l [1 μg/dl = 0.035 μmol/l]) than of controls; cases were four times more likely to have serum retinol levels <0.70 μmol/l and almost three times more likely to have serum retinol levels <1.05 μmol/l (Table 2). A single case who had treated herself with vitamin A and whose serum retinol was 3.09 μmol/l was excluded from this analysis. Mean β-carotene levels were extremely low in both cases (0.22 μmol/l) and controls (0.24 μmol/l) and did not vary significantly by case-status. A large proportion of women in both groups (46% of cases and 26% of controls) had β-carotene levels that were undetectable (<0.035 μmol/l). However, cases were three times more likely to have undetectable β-carotene levels than controls.

Women with XN were more likely to have low anthropometric status reflected by lower body weight (–2.57 kg), BMI (–0.79), MUAC (–0.88 cm), and constituent upper arm muscle (–182 mm^2) and fat (–114 mm^2) areas (Table 3). While cases were 1.3 cm shorter in height than controls, this difference was not statistically significant. Haemoglobin levels among cases were lower, on average by 0.67 g/dl, and three times more likely to be <7 g/dl (representing severe anaemia) than controls (95% CI : 1.25–7.23).

Cases were 2–3 times less likely to have consumed both preformed and carotenoid sources of vitamin A in the past week

13

Table 2 Serum retinol and β-carotene levels of cases and controls

	Case (n = 85) Mean (SD)	Control (n = 90) Mean (SD)	Mean diff.[a] (n = 107 pairs)	SE	*P*-value[b]
Retinol (μmol/l)	0.72 (0.41)	1.03 (0.39)	−0.30	0.05	<0.001
β-carotene (μmol/l)	0.22 (0.37)	0.24 (0.37)	−0.02	0.04	NS
	Case (%)	**Control (%)**	**Matched odds ratio**		**95% CI**
Serum retinol (<0.7 μmol/l)	51.8	21.0	4.0[c]		(2.17–7.37)
(<1.05 μmol/l)	76.8	56.1	2.5[c]		(1.37–4.55)
β-carotene (undetectable)	46.0	26.3	3.3[c]		(1.65–6.72)

[a] Case-control.

[b] Using paired t-test.

[c] Identifies odds ratios with 95% CI that exclude 1.0.

Table 3 Matched mean difference in anthropometric and haemoglobin measurements between cases and controls

	Case Mean (SD)	Control Mean (SD)	Mean Diff.[a]	SE	*P*-value[b]
Weight (kg)	45.2 (5.2)	47.7 (5.0)	−2.57	0.63	<0.001
Height (cm)	149.6 (5.2)	151.0 (5.9)	−1.30	0.72	<0.08
Body mass index (wt/ht^2)	20.2 (1.9)	20.9 (1.8)	−0.79	0.22	<0.001
Mid upper arm circumference (cm)	21.8 (2.1)	22.7 (1.6)	−0.88	0.23	<0.001
Triceps skinfold (mm)	8.7 (2.8)	9.5 (2.6)	−0.84	0.34	<0.02
Muscle area (mm^2)	2919 (479)	3102 (412)	−182	56	<0.002
Fat area (mm^2)	902 (346)	1014 (300)	−114	40	< 0.005
Haemoglobin (g/dl)	8.9 (2.1)	9.6 (1.6)	−0.67	0.22	<0.004

[a] Case-control.

[b] Using paired t-test.

than controls (Figure 1). The relationship was apparent throughout the year, but strongest and statistically significant only in the summer/monsoon months of April–September. In the winter months of October–March, cases and controls were equally likely to have consumed meat and fish sources of vitamin A but in the summer, cases were less likely to eat these foods than controls. Milk and milk products (whey and cream) and provitamin A sources (DGLV and mango) were protective year-round but significantly so only in summer. The protective effect was mainly driven by milk and DGLV consumption.

Seven-day morbidity histories obtained at the time of the XN episode in cases (but during a corresponding gestational time in controls) showed that cases were 2–3 times more likely to report symptoms of urinary/reproductive tract infections that included lower abdominal pain, vaginal discharge and painful and burning urination, diarrhoea/dysentery, and symptoms of nausea, vomiting or poor appetite than controls (Table 4). These symptoms were reported more frequently by cases than controls earlier in pregnancy; ~10 weeks prior to cases developing XN. Women who went on to become cases were also more likely to have reported symptoms of eclampsia (convulsions) or pre-eclampsia (swelling of hands or face) in the previous week (OR = 2.51, 95% CI : 1.13–5.67).

Multivariate conditional logistic regression analysis that included in the model all four classes of risk factors associated with XN, namely diet (weekly vitamin A food intake, using milk and dark green leaves—the two foods that had the most consistent protective effect against XN), protein-energy nutritional

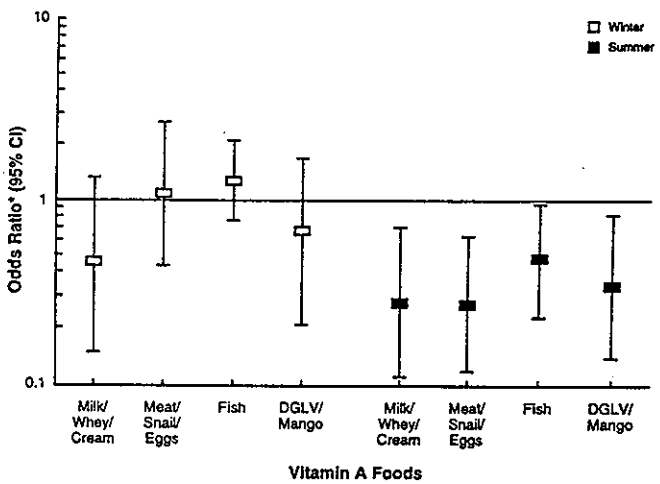


Figure 1 Odds of consuming combinations of foods rich in vitamin A in the past week by season among cases of maternal night blindness relative to non-night blind pregnant controls

* Odds ratios adjusted for mid upper arm circumference and socioeconomic scale using conditional logistic regression.

DGLV—dark green leafy vegetables.

status (MUAC), anaemia (Hb), and morbidity (positive weekly history of lower abdominal pain, diarrhoea/dysentery or nausea) as independent variables, showed that OR associated with each class of factors were significant after adjusting for each others'

14

Table 4 Prevalence of morbidity symptoms in the previous week among cases and controls

Morbidity	During night blindness			Prior to night blindness[a]		
	Case (%)	Control(%)	OR[b]	Case (%)	Control (%)	OR[b]
Urinary/reproductive tract infections[c]	64.6	44.4	2.14[d]	42.6	25.6	1.90[d]
Diarrhoea/dysentery	20	5.2	3.45[d]	5.2	5.9	0.57
Pre-eclampsia/eclampsia[e]	39.1	29.7	1.66	27.8	17.1	2.51[d]
Nausea/ vomiting/ poor appetite	48.2	28.7	2.10[d]	33.9	19.7	2.67[d]

[a] Morbidity history collected ~10 weeks prior to cases developing night blindness.

[b] Odds ratios (OR) adjusted for mid upper arm circumference, socioeconomic scale and age of the woman using conditional logistic regression.

[c] Any lower abdominal pain, painful urination or vaginal discharge.

[d] Identifies OR with 95% CI that exclude 1.0.

[e] Any convulsions or swelling of hands/face.

Table 5 Relative contribution of weekly diet (vitamin A food intake), acute protein-energy malnutrition (mid upper arm circumference [MUAC]), anaemia (haemoglobin [Hb]) and morbidity as risk factors for night blindness during pregnancy

Independent variables	Odds ratio[a]	95% CI
Vitamin A-foods[b] (1–3x)	0.56	(0.24–1.31)
(≥4x)	0.19[c]	(0.08–0.49)
Morbidity[d]	3.28[c]	(1.69–6.34)
MUAC (cm)	0.77[c]	(0.63–0.94)
Hb (g/dl)	0.79[c]	(0.64–0.97)
Socioeconomic scale (≥2)	0.66	(0.32–1.34)

[a] Adjusted using conditional logistic regression.

[b] Vitamin A foods: frequency of milk or dark green leaves consumption in the past week.

[c] Identifies odds ratios with 95% CI that exclude 1.0.

[d] Morbidity: any lower abdnominal pain, diarrhoea/dysentery or nausea in the past week during XN episode in cases.

effects (Table 5). Removing any one of the variables from the model left virtually unchanged the OR estimates for the other covariates, indicating that all four risk factors were independently and strongly associated with XN in pregnant women.

Discussion

Night blind pregnant women were vitamin A-deficient, having, on average, a serum retinol concentration ~33% (0.30 μmol/l) lower than non-night blind controls of similar gestational age. Half of the cases had serum retinol levels <0.70 μmol/l compared to only one-fifth of controls. On matched-analysis, cases had a fourfold higher risk of hyporetinaemia than controls. Although serum β-carotene levels were exceedingly low in both groups of women, the odds of an undetectable level was threefold higher in cases relative to controls. A heightened risk of vitamin A deficiency with XN is fully consistent with experimental data showing vitamin A depletion to cause retinal rod cell dysfunction in animals,[16] impaired dark adaptation in adults,[17,18] and clinical studies in children showing a high risk of hyporetinaemia with XN, and rapid and definitive cure or effective prevention with vitamin A supplements.[19] However, excessive risk of XN persisted in pregnant women at serum retinol levels of 0.70–1.05 μmol/l, suggesting that symptoms can arise in the presence of physiologically significant and acceptable levels of circulating retinol. This suggests that the standard cutoff of <0.70 μmol/l specified by the World Health Organization, as applied typically to children,[20] may be inappropriate for pregnant women. Notably, classic, controlled depletion studies in adults revealed impaired dark adaptation at serum vitamin A levels of 0.70–1.05 μmol/l[17,18] and, in Indonesia, ~20% of vitamin A-responsive night blind children had initial serum retinol levels >0.70 μmol/l.[21]

The relatively late gestational appearance of XN (~7 months) is consistent with the small additional requirement for vitamin A by women confined mostly to the third trimester.[22] Maternal plasma retinol typically decreases during pregnancy[23–27] due to plasma volume expansion. However, in poor, undernourished populations this decline may reflect more than normal haemodilution. Women habitually consuming basal amounts or less of vitamin A are likely to have very poor reserves, and in the absence of dietary improvement during pregnancy, may fail to meet the additional requirements of pregnancy, particularly during the third trimester. Physiological changes combined with a high risk of infection could precipitate acute vitamin A deficiency and lead to XN late in pregnancy.

Other conditions, however, may increase the risk of gestational XN. Protein-energy malnutrition (PEM) could contribute to XN by reducing retinol-binding protein synthesis and thereby decreasing the transport of retinol from the liver into circulation.[28,29] It could also lower synthesis of opsin—the protein moiety of the rod photoreceptor pigment (rhodopsin) in the retina—which could lead to impaired dark adaptation.[16] Night blind women in Nepal were more severely malnourished than their controls by all anthropometric indicators. Elsewhere, low protein-energy status has been associated with hyporetinaemia in pregnant Indonesian,[30] Nigerian[31] and Navajo[32] women, though not to a level that manifested XN. In India, mildly xerophthalmic pregnant women had lower mean weight (43 versus 51 kg) and height (146 versus 153 cm) than non-xerophthalmic ones.[33] A deficiency in zinc, which is required for dark adaptation,[34,35] could also explain the occurrence of XN in the presence of adequate vitamin A status in populations such as rural Nepal, where intakes of meat and fish (sources of zinc) are low and dietary levels of inhibitors of zinc absorption (e.g. phytates from grains) are high.[36] Interim findings of the large trial in Nepal suggest incomplete efficacy of weekly, low-dose vitamin A supplementation in preventing XN in pregnancy (Christian *et al.*, unpublished data).

15

Night blind women consumed vitamin A-rich foods (e.g. milk, snail, fish, dark green leaves, etc.) less frequently than controls late in pregnancy when XN was apparent, especially in the premonsoon and monsoon 'summer' (April–September). Preliminary observations suggest that the occurrence of XN is highest during the summer months, when the number of pregnant women in the last trimester also peaks. Lower availability and higher prices of some of these foods during this time of year may partly explain the seasonally decreased intake.

Cases were at higher risk of anaemia than controls, supporting previous evidence that vitamin A deficiency contributes to low haemoglobin levels.[19] Xerophthalmic pregnant women in India had lower haemoglobin levels (8.3 g/dl) than their non-deficient peers (10.8 g/dl),[33] similar to the differences observed in our study (8.9 versus 9.6 g/dl). The association could be indirect, paralleling a poorer overall nutritional status of night blind women, but could also be causal, illustrated by a significant haemoglobin response to vitamin A supplementation observed in pregnant Indonesian women.[37]

Night blind women were more susceptible to illness, both infectious (e.g. symptoms of urinary and possibly reproductive tract infections, diarrhoea and dysentery) and non-infectious (e.g. symptoms of pre-eclampsia or eclampsia, and poor appetite, nausea and vomiting). Cases were also at greater risk than controls of morbidity (except for diarrhoea/dysentery) when they were assessed 2–3 months earlier in pregnancy; a time that preceded their episode of XN. This estimate of antecedent risk lacks any bias that otherwise could attend morbidity ascertainment at the time cases were night blind. It, thus, could represent effects of subclinical vitamin A deficiency, other predisposing nutritional deficiencies and health risks, as well as, possible (bi-directional) effects of early pregnancy morbidity on later vitamin A status. Vitamin A deficiency has been a long established risk factor for infection, and *vice versa*, especially with respect to persistent diarrhoea and measles in children[19] but evidence of this association in pregnant women has been scant except among HIV-infected subjects.[38,39] Pre-eclampsia or toxaemia in pregnancy has been occasionally associated with vitamin A deficiency and night blindness.[1,26,40] Stronger evidence has emerged in recent years linking low circulating levels of β-carotene,[41,42] a known antioxidant, and serum vitamin A[42] to pre-eclampsia. Infection has only infrequently been associated with vitamin A deficiency in women. Bacteriuria, suggestive of urinary tract infection, was observed in ~80% of pregnant women presenting with nyctalohemeralopia.[43] Lowered vitamin A status during pregnancy has also been shown to increase susceptibility to reproductive tract infection in the puerperium in US women,[44] while a higher vitamin A intake has been linked to a decreased risk of diarrhoea in Bangladeshi women.[45]

We conclude that night blind pregnant women in rural South Asia are exposed to an excess risk of vitamin A deficiency, a known proximal determinant of this condition. In addition, and independent of their vitamin A status, night blind women may be anaemic, acutely malnourished with respect to protein, energy and (likely) other micronutrients such as zinc, and prone to increased risk of infectious and non-infectious illnesses throughout pregnancy. To our knowledge, this is the first population-based report of increased risk of morbidity associated with XN during pregnancy. A low social standing and poverty appear to contribute to this multifaceted syndrome. However, a simple history of maternal XN may help to identify high-risk pregnancies and should be included in antenatal assessment and care programmes on the Indian subcontinent. Based on this evidence, treatment for XN in pregnancy should include vitamin A (although treatment regimens have not been established and efficacy may only be partial). In addition, night blind women may require additional nutritional and antenatal care not previously considered.

Acknowledgements

We thank members of the Nepal Nutrition Intervention Project —Sarlahi in Nepal (beyond the authors) for their assistance in the conduct of this study, especially DN Mandal, TR Shakiya, G Subedi, U Shankar, A Bhetwal, DB Khadka, SV Shrestha, K Dhakal, I Shrestha, and Drs Margaret E Bentley and Rebecca J Stoltzfus for their input and discussion of the results. The study was funded through Cooperative Agreement No DAN 0045-A-00-5094-00 between the Center for Human Nutrition, Department of International Health and the Dana Center for Preventive Ophthalmology, the Johns Hopkins University, Baltimore, MD, USA and the Office of Health and Nutrition, US Agency for International Development, Washington DC, with additional support from Task Force Sight and Life (Roche, Basel, Switzerland).

References

1 Mandal GS, Nanda KN, Bose J. Night blindness in pregnancy. *J Obstet Gynecol India* 1969;**19**:453–58.

2 Dixit DT. Night blindness in third trimester of pregnancy. *Indian J Med Res* 1966;**54**:791–95.

3 Katz J, Khatry SK, West KP Jr *et al.* Night blindness is prevalent during pregnancy and lactation in rural Nepal. *J Nutr* 1995;**125**:2122–27.

4 Starbuck ES. *Night Blindness During Pregnancy as a Risk Marker for Early Infant Death in Jumla, Nepal* (dissertation). Johns Hopkins University, Baltimore, Maryland, 1993.

5 Malyavin A, Beauphanny V, Arouny A, Cohen N. *National Vitamin A Survey in Lao PDR.* Report of the XVII International Vitamin A Consultative Group meeting. Guatemala City, Guatemala. 18–22 March 1996, International Life Sciences Institute, Washington DC, 1996.

6 Linehan M, Shrestha A, Ban B, Rycus P. *Xerophthalmia Prevalence in Ten Districts of the Far and Mid-Eeastern Regions of Nepal.* Report of the XVII International Vitamin A Consultative Group meeting. Guatemala City, Guatemala. 18–22 March 1996, International Life Sciences Institute, Washington DC, 1996.

7 Christian P, Bentley ME, Pradhan R, West KP Jr. An ethnographic study of night blindness ("Ratauri") among women in the Terai of Nepal. *Soc Sci Med*, 1998 (In Press).

8 West KP Jr., Pokhrel RP, Katz J *et al.* Efficacy of vitamin A in reducing preschool child mortality in Nepal. *Lancet* 1991;**338**:67–71.

9 Pokhrel RP, Khatry SK, West KP Jr *et al.* Sustained reduction in child mortality with vitamin A in Nepal (letter) *Lancet* 1994;**342**:1368–69.

10 Katz J, West KP Jr, Khatry SK *et al.* Impact of vitamin A supplementation on prevalence and incidence of xerophthalmia in Nepal. *Invest Ophthalmol Vis Sci* 1995;**36**:2577–83.

11 Brilliant LB, Pokhrel RP, Grasset NC *et al.* Epidemiology of blindness in Nepal. *Bull World Health Organ* 1985;**63**:375–86.

12 Upadhyay MP, Gurung BJ, Pillai KK, Nepal BP. Xerophthalmia among Nepalese children. *Am J Epidemiol* 1985;**121**:71–77.

16

[13] Gibson RS. *Principles of Nutritional Assessment.* New York: Oxford University Press, 1990.

[14] Schlesselman JJ. *Case Control Studies. Design, Conduct Analysis. Monographs in Epidemiology and Biostatistics.* Oxford: Oxford University Press, 1982.

[15] Guttman L. The basis for scalogram analysis. In: *A Source Book for Behavioral Sciences.* Chicago: Aldine, 1974, pp.142–71.

[16] Dowling JE, Wald G. Vitamin A deficiency and night blindness. *Proc Natl Acad Sci* 1958;**44**:648–61.

[17] Hume EM, Krebs HA. *Vitamin A Requirement of Human Adults: An Experimental Study of Vitamin A Deprivation in Man.* Vitamin A subcommittee on the Accessory Food Factors Committee. Medical Research Council, Special Report Series No 264, 1949, pp.7–145.

[18] Sauberlich HE, Hodges RE, Wallace DL *et al.* Vitamin A metabolism and requirements in the human studies with the use of labeled retinol. *Vitam Horm* 1974;**32**:251–75.

[19] Sommer A, West KP Jr. *Vitamin A Deficiency. Health, Survival and Vision.* Oxford: Oxford University Press, New York, 1996.

[20] World Health Organization. *Indicators for Assessing Vitamin A Deficiency and Their Application in Monitoring and Evaluating Intervention Programmes.* Micronutrient Series. WHO, 1996.

[21] Sommer A, Hussaini G, Muhilal, Tarwotjo I, Susanto D, Saroso JS. History of night blindness: a simple tool for xerophthalmia screening. *Am J Clin Nutr* 1980;**33**:887–91.

[22] Underwood BA. Maternal vitamin A status and its importance in infancy and early childhood. *Am J Clin Nutr* 1994;**59(Suppl.)**:517S–24S.

[23] Pulliam RP, Dannenberg WN, Burt RL, Lenke NH. Carotene and vitamin A in pregnancy and early puerperium. *Proc Soc Exp Bio Med* 1962;**109**:913–16.

[24] Venkatachalam PS, Belavady B, Gopalan C. Studies on vitamin A nutritional status of mothers and infants in poor communities in India. *Trop Pediatr* 1962;**61**:262–68.

[25] McGanity WJ, Little HM, Fogelman A, Jennings L, Calhoun E, Dawson EB. Pregnancy in the adolescent. 1. Preliminary summary of the health status. *Am J Obstet Gynecol* 1969;**103**:773–88.

[26] Basu RJS, Arulanantham R. A study of serum protein and retinol levels in pregnancy and toxemia of pregnancy in women of low socioeconomic status. *Indian J Med Res* 1973;**61**:589–95.

[27] Jayasekera JPDJS, Sunethra-Atukorala TM, Seneviratne HR. Vitamin A status of pregnant women in five districts of Sri Lanka. *Asia-Oceania J Obstet Gynaecol* 1991;**17**:217–24.

[28] Smith FR, Goodman DS, Zaklama M, Gahr MK, Maraghy SE, Patwardhan VN. Serum vitamin A, retinol-binding protein, and prealbumin concentrations in protein-calorie malnutrition. I. A functional defect in hepatic retinol release. *Am J Clin Nutr* 1973;**26**:973–81.

[29] Goodman DS. Vitamin A transport and retinol-binding protein metabolism. *Vitam Horm* 1974;**32**:167–80.

[30] Kusin JA, Kardjati S, Suryohudhoyo P, DeWitt C. Anemia and hypovitaminosis A among rural women in east Java, Indonesia. *Trop Geogr Med* 1980;**32**:30–39.

[31] Okolo AA, Omene JA, Glew RH, Diven WF, Warty VS. Vitamins A and E, β-carotene, and proteins in malnourished Nigerian mothers and their newborn infants. *Nutr Res* 1989;**9**:831–38.

[32] Butte NF, Calloway DH, Van Duzen JL. Nutritional assessment of pregnant and lactating Navajo women. *Am J Clin Nutr* 1981;**34**:2216–19.

[33] Shirali GS, Oelbr DG, Mehta KP. Maternal-neonatal serum vitamin A concentrations. *J Pediatr Gastroenterol Nutr* 1989;**9**:62–66.

[34] Vallee BL, Hoch FL. Zinc in horse liver alcohol dehydrogenase. *J Biol Chem* 1957;**225**:185–95.

[35] Huber AM, Gershoff SN. Effects of zinc deficiency on the oxidation of retinol and ethanol in rats. *J Nutr* 1975;**105**:1486–90.

[36] Moser PB, Reynolds RD, Achary S, Howard MP, Andon MB, Lewis SA. Copper, iron, zinc and selenium dietary intake and status of Nepalese lactating women and their breast-fed infants. *Am J Clin Nutr* 1988;**47**:729–34.

[37] Suharno D, West CE, Muhilal, Daryadi D, Hautvast JGAJ. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. *Lancet* 1993;**342**:1325–28.

[38] Semba RD, Miotti PG, Chiphangwi JD *et al.* Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. *Lancet* 1994;**343**:1593–97.

[39] Phuapradit W, Chaturachinda K, Taneepanichskul S, Sirivarasry J, Khupulsup K, Lerdvuthisopon N. Serum vitamin A and β-carotene levels in pregnant women infected with human immunodeficiency virus-1. *Obstet Gynecol* 1996;**87**:564–67.

[40] Koskinen T, Valtomen P, Lehtovaara I, Tuimala R. Amniotic fluid retinol concentration in pregnancy. *Biol Neonate* 1986;**49**:81–84.

[41] Mikhail MS, Anyaegbunam A, Garinkel D, Palan PR, Basu J, Romney SL. Pre-eclampsia and antioxidant nutrients: Decreased plasma levels of reduced ascorbic acid, α-tocopherol, and β-carotene in women with pre-eclampsia. *Am J Obstet Gynecol* 1994;**171**:150–57.

[42] Ziari SA, Mireles VL, Cantu CG *et al.* Serum vitamin A, vitamin E, and β-carotene levels in pre-eclamptic women in Northern Nigeria. *Am J Perinatol* 1996;**13**:287–91.

[43] Edmund C, Clemmesen SV. On parenteral vitamin A treatment of dysaptatio (nyctalo-hemeralopia) in some pregnant women. *Acta Medica Scand* 1936;**89**:69–92.

[44] McGanity WJ, Cannon RO, Bridgforth EB *et al.* The Vanderbilt cooperative study of maternal and infant nutrition. IV. Relationship of obstetric performance to nutrition. *Am J Obstet Gynecol* 1954;**67**:501–27.

[45] Bloem MW, Huq N, Gorstein J *et al. Does the Production of Dark Green Leafy Vegetables and Fruits Play a Role in the Etiology of Maternal Night Blindness, Diarrhea and Malnutrition in Bangladesh.* Report of the XVII International Vitamin A Consultative Group meeting. Guatemala City, Guatemala. 18–22 March 1996, International Life Sciences Institute, Washington DC, 1996.

17

Hyporetinolemia, illness symptoms, and acute phase protein response in pregnant women with and without night blindness[1–3]

Parul Christian, Kerry Schulze, Rebecca J Stoltzfus, and Keith P West Jr

ABSTRACT We examined the association among elevations in acute phase proteins, reported illness, and hyporetinolemia in 234 pregnant Nepali women with (cases) and without (controls) night blindness. Serum α_1-acid glycoprotein (AGP) and C-reactive protein (CRP) were inversely associated with serum retinol concentrations. Elevations in the concentration of CRP in both cases and controls and of AGP in cases were associated with significant reductions (≈0.2–0.3 μmol/L) in serum retinol. The risk of a low serum retinol concentration (< 0.7 μmol/L) with elevated AGP (≥ 1 g/L) and CRP (≥ 5 mg/L) concentrations was significantly higher in cases (odds ratios = 8.6 and 4.3, respectively) than in controls (odd ratios = 1.9 and 2.4, respectively). A 7-d morbidity history indicated that cases were significantly more likely than controls to report symptoms of infections of the urinary, reproductive, and gastrointestinal tracts. Only a few of these symptoms (diarrhea, nausea, and vomiting) were significantly associated with low serum retinol concentrations. Illness in the previous week and elevated CRP or AGP concentrations were synergistically associated with lower serum retinol. For example, the reduction in serum retinol in women with diarrhea and elevated AGP was 0.54 μmol/L, compared with a reduction of 0.03 μmol/L in those with diarrhea only. AGP and CRP may provide useful information about the effect of reported illness on hyporetinolemia in pregnancy. Infection-related hyporetinolemia may predispose women to night blindness during pregnancy in Nepal. *Am J Clin Nutr* 1998;67:1237–43.

KEY WORDS Night blindness, acute phase proteins, morbidity, hyporetinolemia, pregnancy, Nepal, retinol, vitamin A, α_1-acid glycoprotein, C-reactive protein, women

INTRODUCTION

The relation between vitamin A deficiency and infection in children appears to be bidirectional, with each increasing the risk of the other. Whereas systemic infections in children increase the risk of subsequent vitamin A deficiency (1, 2), vitamin A status can alter the severity of morbidities such as diarrhea, measles, and urinary tract infections (3). Histopathologic changes of the epithelium as well as immune depression may be the mechanism by which infection is increased in vitamin A deficiency.

Serum retinol concentrations decline in a variety of infections, including chicken pox (4), malaria (5–7), and measles (2, 8–11), and in diarrheal morbidity (12, 13). The mechanism of lowered serum retinol during an infection may be related to a transient reduction in the production of retinol binding protein (14), a loss of holo–retinol binding protein in the urine (15, 16) and in extracellular fluid (7), and an increased uptake of retinol by certain tissues (17). These responses may be set in motion by proinflammatory cytokines, which are produced early in response to the infectious stimulus (18, 19) and which in turn initiate the acute phase response characterized by the enhanced production of serum proteins such as α_1-acid glycoprotein (AGP) and C-reactive protein (CRP) by the liver. Positive acute phase proteins are defined as proteins whose concentrations increase by ≥25% during infection, inflammation, or trauma (18–20).

Elevations in serum acute phase proteins have been used to indicate the presence of subclinical infection. Serum retinol and positive acute phase proteins have been shown to be negatively correlated in Ghanaian children (5). In this study an elevation in AGP was associated with a 24% decrease in mean serum retinol. This suggests that when serum retinol is used to assess vitamin A status, it may be important to characterize the acute phase protein response in individuals because infection-induced hyporetinolemia may affect the interpretation of serum retinol values.

The relation between infection and vitamin A status in pregnancy is unclear and the available evidence is primarily cross-sectional. Puerperal morbidity in US women was associated with lower vitamin A intakes as well as lower serum vitamin A concentrations throughout pregnancy (21). In Indian women with toxemia, mean serum retinol and carotene concentrations were lower than in healthy women in their third trimester of pregnancy (22). Cross-sectional data show that HIV-1–infected pregnant women who were vitamin A deficient were more likely to pass on the infection to their infants than were infected women who had higher serum retinol concentrations (23).

[1] From the Center for Human Nutrition, Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore.

[2] Supported by Cooperative Agreement no. DAN 0045-A-00-5094-00 between the Center for Human Nutrition, Department of International Health, The Johns Hopkins University, Baltimore, and the Office of Health and Nutrition, US Agency for International Development, Washington, DC, with additional support from Task Force Sight and Life (Roche, Basel, Switzerland).

[3] Address reprint requests to P Christian, Division of Human Nutrition, Johns Hopkins School of Hygiene and Public Health, 615 North Wolfe Street, Room 2041, Baltimore, MD 21205. E-mail: pchristi@jhsph.edu.

Received August 15, 1997.

Accepted for publication November 6, 1997.

The American Society for Clinical Nutrition Inc. does not endorse any commercial enterprise.

B

We conducted a population-based, case-control study of the risk factors of night blindness during pregnancy in the southeastern plains of Nepal (24), where the prevalence of this condition is reported to be high (25). The mean serum retinol concentration was lower by 0.30 μmol/L and the risk of being hyporetinolemic (serum retinol < 0.7 μmol/L) was four times greater (95% CI: 2.2, 7.4) in cases than in pregnant controls without night blindness (24). However, women with night blindness were also more likely to have been ill, suggesting that morbidity may have contributed to their low serum retinol concentrations. The role of infection as an underlying cause of vitamin A deficiency may not be recognized if information on morbidity is not taken into account. In this paper we present *1*) the association between serum retinol concentrations and concentrations of acute phase proteins (AGP and CRP) in women with and without night blindness, *2*) the relation between night blindness and reported history of morbidity in the previous week, *3*) the association of morbidity with elevations in acute phase proteins and hyporetinolemia, and *4*) the influence of reported morbidity symptoms when associated with an acute phase protein response on low serum retinol concentration.

SUBJECTS AND METHODS

A population-based case-control study was conducted in 1994–1995 to examine risk factors of night blindness during pregnancy in Nepal. Ethical approval was obtained from the Nepal Health Research Council and the Johns Hopkins Committee on Human Research. The case-control study was nested within a randomized, double-masked, maternal vitamin A and β-carotene supplementation trial being conducted in the Sarlahi district of Nepal. The supplementation trial was designed to assess the effect of supplementation on fetal, infant, and maternal mortality. Weekly dosing for this trial was done by female distributors who also identified new pregnancies and women with night blindness through a weekly assessment of the history of night blindness in the study area. A woman's history of night blindness was verified by probing further for symptoms of poor vision at dusk and at night. Only women who had poor or no vision at night but could see clearly during the day were enrolled as cases in the study. Controls, matched for gestational month of pregnancy, were picked randomly from a pool of all other pregnant women without night blindness in the study area. A home-based assessment of cases and controls was done within 1 wk of enrollment. The assessment included collecting a 7-d history of the frequency of food intake and morbidity, taking anthropometric measurements, and drawing capillary blood for biochemical analyses. Over a period of 15 mo, 117 pairs of cases and controls were enrolled in the study.

In the morbidity recall, women were asked the number of days they suffered from 19 different symptoms in the past week. Only 13 symptoms are reported here because the proportions of women reporting the other 6 symptoms were < 5%. Anthropometric assessment included measurement of weight, height, midupper arm circumference, and triceps skinfold thickness with methods described by Gibson (26). Blood was collected in Microtainer collection tubes (Becton-Dickinson, Franklin Lakes, NJ) and stored on ice in the field and centrifuged at 1530 × *g* for 10 min at room temperature within 4–5 h of collection; the serum obtained was stored and transported in liquid nitrogen to the Center for Human Nutrition Laboratory of the Johns Hopkins University in Baltimore. Once in Baltimore, the serum samples were stored at −70° until analyzed.

Serum analysis

Retinol was analyzed by reversed-phase HPLC with a Beckman System Gold column (Beckman, Columbia, MD), a 110 pump system, a Beckman 167 detector, and a 710B Waters Autosampler (Waters, Milford, MA). The mobile phase was 83% acetonitrile, 13% dioxane, 4% methanol with 0.15 mol ammonium acetate/L added to the methanol, and 0.1% triethylamine. The detector wavelengths were 450 nm for β-apo-8′-carotenal (internal standard) and 325 nm for retinol and retinyl esters.

Retinol was extracted by using ethanol and hexane solvents and the residue allowed to dissolve in 100 μL of the mobile phase. A portion (20 μL) of the sample was injected into the HPLC system. The concentration of retinol was calculated by the ratio of the internal standard to that of the sample by using the areas under the peak on the chromatogram. Known concentrations of pooled serum samples were run with the unknown samples simultaneously. A standard reference material (SRM 968b) of known retinol concentration from the Standard Reference Material Program of the National Institute of Standards and Technology (Gaithersburg, MD) was used for standardizing the assay.

Serum AGP was analyzed with a competitive, time-resolved, fluorescent immunoassay method based on the enzyme-linked immunosorbent assay method reported by Filteau et al (27). A 1:8000 dilution of anti-AGP (Dako Corp, Carpinteria, CA) in bicarbonate buffer, pH 9.6, was bound to microwells. Then 25 μL of standards (Behring Diagnostics Inc, Westwood, MA) and samples, diluted 1:1000 in tris-HCL buffer, pH 7.8, was added to each microwell with an equal volume of biotinylated AGP diluted 1:8000 in tris-HCL buffer, pH 7.8. After a 2-h incubation, europium-labeled streptavidin (Wallac Inc, Gaithersburg, MD) was added to each microwell. The fluorescence of each well was determined in an Arcus fluorometer (Wallac) and standard curves were developed for each microplate. Biotinylated AGP was prepared by incubating purified AGP (Sigma, St Louis) in a sodium acetate–sodium periodate solution, pH 4.5, in a ratio of 2 g/L. Excess periodate was removed with a PD-10 column, and biotin hydrazide was added and the mixture incubated at 37 °C for 2 h. Excess biotin was removed by dialyzing the solution overnight in tris-buffered saline.

Serum CRP was determined by using a commercial enzyme-linked immunosorbent assay kit (Hemagen Diagnostics, Waltham, MA). Standards, controls, and serum were diluted (1:101) and pipetted into microwells bound with goat anti-human-CRP. Rabbit anti-CRP conjugated with horseradish peroxidase was used as the second antibody. On activation with 3,3′,5,5′-tetramethylbenzidine, plates were read at 450 nm. Serum retinol, AGP, and CRP values were available for 226, 223, and 221 of 234 women, respectively. Missing values were either due to a subject's refusal to give blood or the collection of insufficient quantities of serum.

Statistical analysis

Geometric means and 95% CIs for AGP and CRP were calculated by performing a logarithmic transformation of the data for analysis. The resulting values are presented here in their original units. The logarithmic transformation was done to normalize the skewed distribution. Student's *t* tests were used to compare mean

serum retinol concentrations stratified by elevated and normal acute phase protein concentrations. Pearson's correlation coefficients were calculated between the log-transformed values of serum retinol and AGP and CRP concentrations. Odds ratios (ORs) and 95% CIs were calculated to examine the association between dichotomized acute phase protein concentrations and low serum retinol concentrations (using <0.7 μmol/L as the cutoff). The cutoffs for elevations in AGP and CRP were set at concentrations of ≥1 g/L (5) and ≥5 mg/L, respectively. The cutoff for CRP was set lower than the more frequently used value of ≥10 mg/L (28) because concentrations of this protein in our study women tended to be lower than expected in populations with high levels of infection. The cutoff of ≥5 mg/L was used previously in a study examining acute phase protein concentrations of healthy, pregnant Norwegian women (29).

To examine univariate differences in dichotomized morbidity status by case-control status, McNemar's matched OR, with the ratio of discordant pairs, and test-based 95% CIs were calculated (30). Chi-square and Fisher's exact statistics (when the cell size was less than five) were calculated to make comparisons between proportions of women with elevated AGP or CRP and low retinol concentrations among asymptomatic women and women with individual symptoms in the previous 7 d.

Multiple linear regression analyses were done with continuous serum retinol concentration as the dependent variable and diarrhea, nausea, fever, and productive cough as explanatory variables. These symptoms were selected because they were significantly associated with serum retinol or acute phase proteins in the bivariate analysis. Vaginal discharge, headache, and dysentery, also found to be significant in the bivariate analysis, were neither significant nor did they change the parameter estimates of the other variables in the model and were therefore excluded in the multivariate analysis. Separate models were run with diarrhea, nausea and vomiting, cough, and fever recorded as indicator variables. These indicator variables were categorized into three groups: *1)* individuals with an illness symptom and a normal acute phase protein concentration, *2)* individuals with the same symptom accompanied by an elevated acute phase protein concentration, and *3)* the reference group, defined as individuals without a history of that symptom. Each model was adjusted for the presence of the other illness symptoms, gestational age at the time of the assessment in weeks, and midupper arm circumference. Results of the multivariate analyses are presented for cases and controls combined because the parameter estimates were nearly identical when analyzed separately. All analyses were performed with SAS/PC+ version 6.11 (SAS Institute Inc, Cary, NC).

TABLE 1
Characteristics of pregnant women with (cases) and without (controls) night blindness

	Cases (n = 117)	Controls (n = 117)
Age (y)	24.9 ± 5.0[1]	24.2 ± 4.9
Gestational age at the time of assessment (wk)[2]	29.7 ± 6.6	30.1 ± 6.9
Previous pregnancies >2 (%)	44.8	47.1
Literate (%)	5.9	12.6
Serum retinol (μmol/L)	0.72 ± 0.41[3]	1.03 ± 0.39

[1] $\bar{x}$ ± SD.
[2] Cases and controls were matched for gestational age in months.
[3] Significantly different from controls, $P < 0.001$.

RESULTS

Cases and controls had similar mean ages and number of previous pregnancies and were assessed at ≈30 ± 7 wk of gestation (**Table 1**). The mean serum retinol concentration was ≈0.3 μmol/L lower in cases than in controls. The geometric mean (and 95% CI) concentrations of acute phase proteins were 0.6 g/L (0.2, 1.6 g/L) for AGP and 2.8 mg/L (0.5, 15.6 mg/L) for CRP in cases and 0.52 g/L (0.2, 1.4 g/L) for AGP and 2.6 mg/L (0.5, 14.4 mg/L) for CRP in controls. The proportions of women with elevated AGP (≥1 g/L) and CRP (≥5 mg/L) concentrations among all women were 13.8% and 18.1%, respectively. The proportion with elevated CRP tended to be higher (although not significantly so) in cases (22%) than in controls (14%), but the rates of elevated AGP were not significantly different (15% compared with 12% in cases and controls, respectively). Acute phase protein concentrations did not vary with gestational age at the time of the assessment ($r = -0.09$ for AGP and 0.08 for CRP; $P \geq 0.05$). Serum retinol concentrations, on the other hand, were negatively correlated with the gestational age of women at the time of assessment ($r = -0.28$, $P < 0.001$).

There was a negative correlation between serum retinol and acute phase proteins that appeared to be stronger with CRP than with AGP. For AGP, the correlation coefficients were −0.17 ($P = 0.079$) and −0.08 ($P = 0.41$) in cases and controls, respectively. For CRP, these values were −0.36 ($P < 0.001$) and −0.33 ($P < 0.001$), respectively. The correlation between serum CRP and AGP concentrations was high (0.48, $P < 0.001$).

The mean serum retinol concentration was significantly lower by 0.2–0.3 μmol/L in cases with elevated AGP and CRP concentrations (**Figure 1**). Among controls, elevated CRP concentrations were associated with a significant reduction in serum retinol by 0.22 μmol/L but elevated AGP concentrations were not significantly associated with serum retinol. Women with elevated AGP or CRP concentrations (cases and controls combined) were ≈3.5 times as likely to have a serum retinol concentration <0.7 μmol/L than women who did not have elevated concentrations of these proteins (**Table 2**). The risk of a low serum retinol concentration associated with elevated AGP and CRP was higher among cases than controls. This difference between cases and

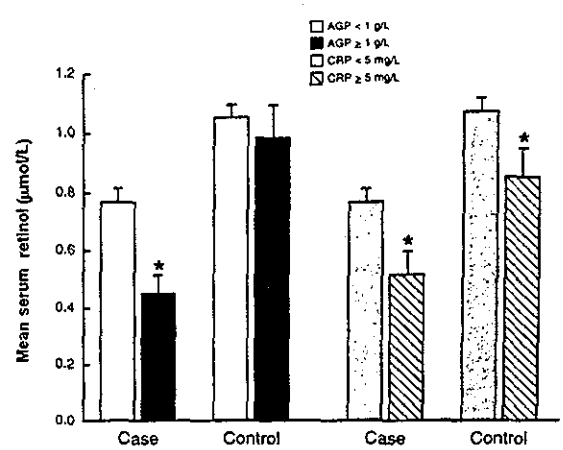


FIGURE 1. Relation between serum concentrations of acute phase proteins and retinol by case-control status (for which cases were pregnant women with night blindness and controls were pregnant women without night blindness). AGP, α_1-acyl glycoprotein; CRP, C-reactive protein. *Significantly lower than in women in whom AGP or CRP concentrations were not elevated (ie, <1 g/L and <5 mg/L, respectively), $P < 0.05$.

20

TABLE 2
Odds ratio (OR) of low and low-to-moderate serum retinol concentrations associated with elevated α_1-acyl glycoprotein (AGP) and C-reactive protein (CRP) concentrations by case-control status

	AGP ≥ 1 g/L	AGP < 1 g/L	OR (95% CI)	CRP ≥ 5 mg/L	CRP < 5 mg/L	OR (95% CI)
	n (%)	*n (%)*		*n (%)*	*n (%)*	
Serum retinol < 0.7 μmol/L						
Cases	15 (88.2)	43 (46.7)	8.55 (2.24,32.7)	18 (78.3)	38 (45.8)	4.26 (1.49, 12.09)
Controls	4 (30.8)	18 (19.2)	1.88 (0.55, 6.48)	5 (33.3)	16 (17.2)	2.41 (0.76, 7.73)
Total	19 (63.3)	61 (32.8)	3.54 (1.60, 7.79)	23 (60.5)	54 (30.7)	3.46 (1.69, 7.09)
Serum retinol < 1.05 μmol/L						
Cases	17 (100.0)	67 (72.8)	6.34 (1.01, 40.03)	20 (86.9)	63 (75.9)	2.12 (0.60, 7.31)
Controls	5 (38.5)	53 (56.4)	0.5 (0.15, 1.52)	10 (66.7)	50 (46.2)	1.72 (0.57, 5.19)
Total	22 (73.3)	120 (64.5)	1.51 (0.65, 3.51)	30 (78.9)	113 (64.2)	2.09 (0.92, 4.74)

controls is not surprising considering that the serum retinol concentrations of the cases were distributed at much lower values (by −0.3 μmol/L) than were those of the controls.

Cases were more likely than controls to have been ill in the previous week (**Figure 2**). Cases were twice as likely to report symptoms of urinary and reproductive tract infections, such as lower abdominal pain and painful and burning urination; two to three times as likely to report nausea, vomiting, and low appetite; and 14 times as likely to report diarrhea (watery stools for ≥4 d). The odds of reporting other morbidity symptoms such as convulsions, vaginal discharge, and dysentery were higher in cases than in controls, but the differences were not significant.

We next examined the association between the women's 7-d morbidity history and elevated proteins and low serum retinol (<0.7 μmol/L) in cases and controls combined (**Table 3**). The reference group was women who were asymptomatic (n = 49; 22%), ie, those who did not report any of the 13 morbidity symptoms in the previous week. Comparisons were made between asymptomatic women and women with any symptoms or with individual symptoms. Significantly more women reporting vaginal discharge, diarrhea, high fever, and productive cough had elevated AGP concentrations than did asymptomatic women. Similarly, CRP concentrations were significantly elevated in women with symptoms of dysentery, high fever, headache, or vomiting in the previous 7 d compared with asymptomatic women. A significantly higher proportion of women had low serum retinol concentrations if they had diarrhea, nausea, or vomiting in the previous week than if they were asymptomatic.

Results of the multivariate regression analyses are presented as estimated serum retinol concentrations in individuals with an illness symptom with and without elevated AGP or CRP concentrations, after other illness symptoms, gestational age in pregnancy, and arm circumference were adjusted for (**Table 4**). After other morbidities were adjusted for, a history of diarrhea was associated with serum retinol concentrations that were ≈0.5 μmol/L lower when AGP or CRP concentrations were elevated than were concentrations in subjects with no history of diarrhea in the previous 7 d. Serum retinol remained virtually unchanged when diarrhea was not accompanied by elevated AGP (0.88 compared with 0.85 μmol/L for subjects with no history of diarrhea and those with a history of diarrhea but without elevated AGP, respectively) or CRP (0.88 compared with 0.77 μmol/L for subjects with no

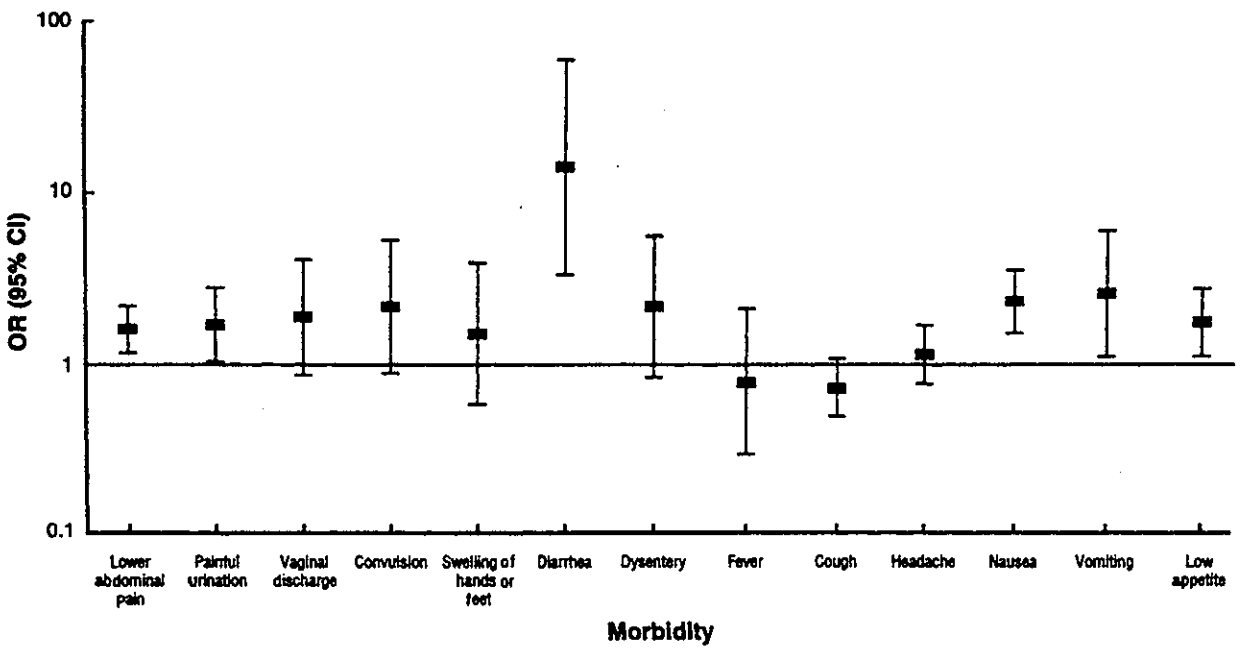


FIGURE 2. Matched odds ratios (ORs) and 95% CIs of reported morbidity in the previous week among cases (pregnant women with night blindness) relative to controls (pregnant women without night blindness).

TABLE 3
Association between reported 7-d morbidity and serum retinol and acute phase protein concentrations[1]

Morbidity history	AGP ≥1g/L	CRP ≥5 mg/L	Retinol <0.7 μmol/L
	n (%)	*n (%)*	*n (%)*
Asymptomatic	3/47 (6.4)	5/49 (10.2)	14/49 (28.6)
Any symptom	27/176 (15.3)	35/172 (20.4)	68/177 (38.4)
Individual symptoms			
Lower abdominal pain	12/100 (12.0)	18/95 (18.9)	43/101 (42.6)
Painful urination	7/51 (13.7)	7/51 (13.7)	16/51 (31.4)
Vaginal discharge	5/22 (22.7)[2]	3/22 (13.6)	9/22 (40.9)
Convulsions	4/19 (21.0)	4/18 (22.2)	7/18 (38.9)
Swelling of hands or feet	3/15 (20.0)	4/15 (26.7)	6/13 (46.2)
Diarrhea	6/15 (40.0)[2]	4/14 (28.6)	10/15 (66.7)[2]
Dystentery	1/16 (6.2)	5/15 (33.3)[2]	8/18 (44.4)
High fever	6/16 (37.5)[2]	7/16 (43.8)[2]	6/16 (37.5)
Productive cough	16/70 (22.9)[2]	15/70 (21.4)	22/74 (29.7)
Headache	9/61 (14.7)	15/60 (25.0)[2]	20/62 (32.3)
Nausea	10/59 (16.9)	13/57 (22.8)	29/60 (48.3)[2]
Vomiting	4/22 (18.2)	7/21 (33.3)[2]	13/25 (52.0)[2]
Low appetite	*5/53 (9.4)*	*10/53 (18.9)*	*25/53 (47.2)*

[1] AGP, α_1-acyl glycoprotein; CRP, C-reactive protein.
[2] Significantly different from asymptomatic women, $P < 0.05$ (chi-square and Fisher's exact tests).

TABLE 4
Estimated serum retinol concentrations by recent disease status among women with and without elevated acute phase protein concentrations[1]

	Serum retinol	
	AGP	CRP
	μmol/L	
Diarrhea		
Absent	0.88 [220]	0.88 [221]
Present without elevated protein	0.85 [9]	0.77 [10]
Present with elevated protein	0.34[2] [6]	0.37[2] [4]
Nausea or vomiting		
Absent	0.91 [173]	1.05 [175]
Present without elevated protein	0.78[2] [51]	0.92[2] [46]
Present with elevated protein	0.46[2] [11]	0.62[2] [14]
Productive cough		
Absent	0.79 [165]	0.79 [165]
Present without elevated protein	1.05[2] [54]	1.06[2] [55]
Present with elevated protein	0.90 [16]	0.81 [15]
Fever		
Absent	0.87 [219]	0.87 [219]
Present without elevated protein	0.85 [10]	0.92 [9]
Present with elevated protein	*0.75 [6]*	*0.64 [7]*

[1] *n* in brackets. Retinol concentrations were adjusted for gestational age (weeks) of pregnancy at the time of assessment, arm circumference, and other illness symptoms. Elevated acute phase protein concentrations were defined as follows: α_1-acyl glycoprotein (AGP) ≥ 1 g/L and C-reactive protein (CRP) ≥ 5 mg/L. Values in the "absent" row are serum retinal concentrations in the reference category, ie, without stratification for elevated acute phase protein concentrations.
[2] Significantly different from women without illness, $P < 0.05$.

history of diarrhea and those with a history of diarrhea but without elevated CRP, respectively) concentrations. A history of nausea or vomiting was associated with a significant decrease in serum retinol of 0.13 μmol/L, or 0.4 μmol/L when accompanied by elevated acute phase proteins. A history of productive cough was associated with a significantly higher serum retinol concentration when not accompanied by increased AGP or CRP concentrations. Fever concomitant with elevated AGP or CRP concentrations was associated with a greater decline in serum retinol than was fever alone, but these differences were not significant.

DISCUSSION

To our knowledge this is the first study that has examined the complex relation between infection, acute phase proteins, and serum retinol with respect to night blindness during pregnancy. There was a weak association between reported illness and elevations in serum acute phase proteins or low serum retinol concentrations. Elevated AGP or CRP concentrations accompanying a recent illness, however, were strongly associated with a reduction in serum retinol, sometimes of as much as 0.5 μmol/L, as for diarrhea. These results suggest that measures of the acute phase response, such as concentrations of AGP and CRP, can provide additional information when serum retinol is evaluted. The influence of morbidity on serum retinol did not differ by case-control status, but because cases were more hyporetinolemic and got sicker more often and more severely, they were more likely to have infection-related decreases in serum retinol.

Serum retinol and acute phase proteins

We found lower CRP values than would be expected in a population with a high prevalence of infection. During acute episodes of illness, concentrations of CRP can become dramatically elevated, peaking as much as 1000 times more than baseline values within 48 h of infection and returning to normal within 1 wk (31). The generally poor nutritional status of this population, as well as the long time period (7 d) for a qualifying morbidity episode, may have attenuated CRP responses (5). This was one of the reasons for choosing the cutoff of 5 mg/L to distinguish elevated CRP concentrations, rather than the more conventional cutoff of 10 mg/L (28). Still, the expected inverse relation between CRP and serum retinol existed for both cases ($r = -0.36$) and controls ($r = -0.33$). In fact, the magnitude of this relation was similar to that found by Filteau et al (5) in Ghanaian children ($r = -0.33$, $P < 0.001$). The negative association of serum retinol with CRP was stronger than with AGP for both cases and controls. This is plausible because CRP, which is elevated to a greater extent during infection, is more reflective of a current or recent episode of illness than AGP. CRP concentrations peak 48 h after infection and then decline, whereas AGP concentrations plateau for ≈120 h after infection (31).

The risk of low serum retinol was ≈3.5 times higher in subjects with elevated AGP or CRP concentrations, a finding also shown by Filteau et al (5) in Ghanaian children. Of interest was the apparently higher risk of low serum retinol (<0.7 μmol/L) in cases than in controls given an elevated AGP (OR = 8.6 compared with 1.9, respectively) or CRP (OR = 4.3 compared with 2.4, respectively) concentration. The overall risk of low serum retinol was, however, four times as high and the mean serum retinol concentration ≈0.3 μmol/L lower in cases than in controls.

Furthermore, reported morbidity was significantly greater in cases than in controls. It is possible that infections were less severe and resolved more quickly in controls than in cases and that as a result serum retinol values returned to normal. The degree of decline in serum retinol is related to the severity and duration of infection (32).

Morbidity and acute phase proteins

Serum AGP and CRP covaried differently with reported illness and serum retinol concentration. Serum AGP concentrations were significantly increased in women who reported vaginal discharge, diarrhea, high fever, or productive cough in the previous week, whereas CRP concentrations were significantly increased in women with reported cough, high fever, severe headache, or vomiting. The different characteristics of the AGP and CRP responses may explain why increases in these proteins were not necessarily associated with the same morbidities. Fever is a well-known sequelae of an acute phase response and it is not surprising that both AGP and CRP concentrations were strongly associated with symptoms of high fever in the past week. Also, the type, severity, and duration of infection may determine the nature of the acute phase response and its characteristics. We found some evidence of a dose-response association between the number of days of reported symptoms and mean concentrations of AGP and CRP. For example, the mean AGP concentration increased from 0.54 g/L in women not reporting fever to 0.69 g/L in those with fever for 1–3 d and to 0.74 g/L in those with fever for 4–7 d ($P = 0.12$).

Morbidity and serum retinol

Morbidity, by reported illness or presence of infection indicated by acute phase protein concentrations, was associated with low serum retinol, although the relation was observed with only a few morbidity symptoms. This association was not found in children in northern Ghana aged 6–59 mo (5). In the Ghanaian study, the mean serum retinol concentration did not differ between children with diarrhea or vomiting and asymptomatic children. Only in children with fever were serum retinol concentrations significantly lower. In contrast, we detected a significant difference in the prevalence of low serum retinol between asymptomatic women and those with diarrhea, nausea, or vomiting. Biological mechanisms that explain the relation between gastrointestinal symptoms and low serum retinol include anorexia, reduced absorption of vitamin A (33), and increased urinary excretion of vitamin A (16). Nausea and vomiting late in pregnancy may result in reduced food intake along with lowered nutrient absorption, although data are scarce on the actual mechanism of this relation.

Serum retinol, acute phase proteins, and morbidity

Multivariate analyses showed that diarrhea and nausea in the past week were associated with a decline in serum retinol, but the decline was largely restricted to women with elevated acute phase protein concentrations. The magnitude of effect of elevated AGP and CRP on serum retinol concentrations in the presence of a diarrheal episode in the past 7 d was striking even after other types of morbidity, gestational age, and arm circumference were controlled for. Women with elevated AGP or CRP concentrations and reporting diarrhea had serum retinol concentrations that were on average 0.5 μmol/L lower than those in women with no diarrhea. Conversely, serum retinol concentrations of women reporting diarrhea but with no elevation in acute phase proteins did not differ from those in women with no diarrhea. Although this appears to suggest that women with an illness accompanied by elevated proteins are more severely ill, it is more plausible that an acute phase response more accurately captures a more recent episode of infection than a morbidity recall of the previous 7 d. Our findings highlight the utility of measuring acute phase proteins to better interpret vitamin A status.

A history of productive cough in the absence of elevated AGP or CRP concentrations was significantly associated with an increase in serum retinol concentrations of ≈0.3 μmol/L over concentrations found in women without a cough. Data from a longitudinal morbidity trial in Central Java found the relative risk of cough and acute lower respiratory tract infection to be higher among well-nourished, vitamin A–supplemented children aged < 1 y than among unsupplemented children (34). It has been hypothesized that increased cough in vitamin A–supplemented children may reflect a recovered ability to generate a cough and clear pathogens from the respiratory airway subsequent to the reversal of keratinizing metaplasia of the respiratory tract and the recovery of mucus-producing epithelium with improved vitamin A status (3). In our study, the vitamin A status of the controls, who were more likely to show symptoms of upper respiratory tract infection, was better than that of the cases as indicated by serum retinol concentrations.

Our findings suggest that AGP or CRP may provide useful information for interpreting serum retinol concentrations in the presence or absence of a symptom of illness. These proteins provide information in addition to that obtained through morbidity recall, which may be inaccurate as a result of interviewer and subject biases. A practical question is whether AGP and CRP are useful independently for measuring an acute phase response. Our data do not provide evidence that AGP and CRP provide mutually exclusive information for the interpretation of serum retinol concentrations in a population. The magnitude of the decrease in serum retinol associated with an acute phase protein response was similar for both CRP and AGP, suggesting that either protein may be equally useful for interpreting serum retinol values. However, cross-sectional data are inadequate for answering this question conclusively and future studies with a prospective design assessing these markers at multiple time points should address this issue.

Infection-related hyporetinolemia was higher in women with night blindness than in women without. This led us to hypothesize that infection-related hyporetinolemia may predispose women to night blindness during pregnancy. For example, an illness episode such as of diarrhea may result in severe hyporetinolemia in an already vitamin A–deplete pregnant woman. The relative odds ratio of diarrhea occurring in cases compared with controls in the present study was 14, clearly indicating a significant risk among women with night blindness of having a recent episode of diarrhea. Our cross-sectional study cannot adequately test this hypothesis, but future studies should investigate the role of infection-induced hyporetinolemia in the etiology of night blindness in women. In populations in whom night blindness is prevalent, subclinical or clinical infection may in part explain the hyporetinolemia associated with this condition.

We thank members of the Nepal Nutrition Intervention Project-Sarlahi for their assistance in the conduct of this study, especially SK Khatry, SR Shrestha, S LeClerq, E Kimbrough-Pradhan, DN Mandal, TR Shakiya, and RP Pokhrel.

REFERENCES

1. Sommer A, Tarwotjo I, Katz J. Increased risk of xerophthalmia following diarrhea and respiratory disease. Am J Clin Nutr 1987;45:977–80.
2. Reddy V, Bhaskaram P, Raghuramulu N, et al. Relationship between measles, malnutrition, and blindness: a prospective study in Indian children. Am J Clin Nutr 1986;44:924–30.

3. Sommer A, West KP Jr. Vitamin A deficiency. Health, survival and vision. New York: Oxford University Press, 1996.
4. Campos FACS, Flores H, Underwood BA. Effect of an infection on vitamin A status of children as measured by the relative dose response (RDR). Am J Clin Nutr 1987;46:91–4.
5. Filteau SM, Morris SS, Abbott RA, et al. Influence of morbidity on serum retinol of children in a community-based study in northern Ghana. Am J Clin Nutr 1993;58:192–7.
6. Tabone MD, Muanza K, Lyagoubi M, et al. The role of interleukin-6 in vitamin A deficiency during *Plasmodium falciparum* malaria and possible consequences for vitamin A supplementation. Immunology 1992;75:553–4.
7. Thurnham DI, Singkamani R. The acute phase protein response and vitamin A status in malaria. Trans R Soc Trop Med Hyg 1991;85:194–9.
8. Markowitz LE, Nzilambi N, Driskell WJ, et al. Vitamin A levels and mortality among hospitalized measles patients, Kinshasa, Zaire. J Trop Pediatr 1989;35:109–12.
9. Frieden TR, Sowell AL, Henning KJ, Huff DL, Gunn RA. Vitamin A levels and severity of measles, New York City. Am J Dis Child 1992;146:182–6.
10. Hussey GD, Klein M. A randomized, controlled trial of vitamin A in children with severe measles. N Engl J Med 1990; 323:160–4.
11. Coutsoudis A, Broughton M, Coovadia HM. Vitamin A supplementation reduces measles morbidity in young African children: a randomized, placebo-controlled, double-blind trial. Am J Clin Nutr 1991;54:890–5.
12. Bloem MW, Wedel M, Egger RJ, et al. Mild vitamin A deficiency and risk of respiratory tract diseases and diarrhea in preschool and school children in northeastern Thailand. Am J Epidemiol 1990; 131:332–9.
13. Salazar-Lindo E, Salazar M, Alvarez JO. Association of diarrhea and low serum retinol in Peruvian children. Am J Clin Nutr 1993;58:110–3.
14. Rosales FJ, Ritter SJ, Zolfaghari R, Smith JE, Ross AC. Effects of acute inflammation on plasma retinol, retinol binding protein, and its mRNA in the liver and the kidneys of vitamin A-sufficient rats. J Lipid Res 1996;37:962–71.
15. Stephensen CB, Alvarez JO, Kohatsu J, Hardmeier R, Kennedy JI Jr, Gammon RB Jr. Vitamin A is excreted in the urine during acute infection. Am J Clin Nutr 1994;60:388–92.
16. Alvarez JO, Salazar-Lindo E, Kohatsu J, Miranda P, Stephensen CB. Urinary excretion of retinol in children with acute diarrhea. Am J Clin Nutr 1995;61:1273–6.
17. Willumsen JF, Simmank K, Filteau SM, Wagstaff LA, Tomkins AM. Toxic damage to the respiratory epithelium induces acute phase changes in vitamin A metabolism without depleting retinol scores of South African children. J Nutr 1997;127:1339–43.
18. Kushner I. Regulation of the acute phase response by cytokines. Perspect Biol Med 1993;36:611–2.
19. Raynes JG. The acute phase response. Biochem Soc Trans 1994; 22:69–74.
20. Kushner I. The phenomenon of the acute phase response. Ann N Y Acad Sci 1982;389:39–48.
21. McGanity WJ, Cannon RO, Bridgeforth EB, et al. The Vanderbilt cooperative study of maternal and infant nutrition. VI. Relationship of obstetric performance to nutrition. Am J Obstet Gynecol 1954;67:501–27.
22. Basu RJS, Arulanantham R. A study of serum protein and retinol levels in pregnancy and toxemia of pregnancy in women of low socio-economic status. Indian J Med Res 1973;61:589–95.
23. Semba RD, Miotti PG, Chiphangwi JD, et al. Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. Lancet 1994; 343:1593–7.
24. Christian P, West KP Jr, Khatry SK, et al. Night blindness of pregnancy in rural Nepal—nutritional and health risks. Int J Epidemiol (in press).
25. Katz J, Khatry SK, West KP Jr, et al. Night blindness is prevalent during pregnancy and lactation in rural Nepal. J Nutr 1995;125:2122–7.
26. Gibson RS. Principles of nutritional assessment. New York: Oxford University Press, 1990.
27. Filteau SM, Morris SS, Tomkins AM, et al. Lack of association between vitamin A status and measures of conjunctival epithelial integrity in young children in Northern Ghana. Eur J Clin Nutr 1994;48:669–77.
28. Young B, Gleeson M, Cripps AW. C-reactive protein: a critical review. Pathology 1991;23:118–24.
29. Haram K, Augensen K, Elsayed S. Serum protein pattern in normal pregnancy with special reference to acute-phase reactants. Br J Nutr 1983;90:139–45.
30. Schlesselman JJ. Case control studies. Design, conduct, analysis. Monographs in epidemiology and biostatistics. Oxford, United Kingdom: Oxford University Press, 1982.
31. Fleck A. Clinical and nutritional aspects of changes in acute phase protein during inflammation. Proc Nutr Soc 1989;48:347–54.
32. Clausen SW, McCord AB. The carotenoids and vitamin A of the blood. J Pediatr 1938;13:635–50.
33. Feachem RG. Vitamin A deficiency and diarrhea: a review of interrelationships and their implications for the control of xerophthalmia and diarrhoea. Trop Dis Bull 1987;84:1–16.
34. Dibley MJ, Sadjimin T, Kjolhede CL, Moulton LH. Vitamin A supplementation fails to reduce incidence of acute respiratory illness and diarrhea in pre-school age Indonesian children. J Nutr 1996;126:434–42.

Pergamon

PII: S0277-9536(97)00214-1

Soc. Sci. Med. Vol. 46, No. 7, pp. 879–889, 1998
© 1998 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
0277-9536/98 $19.00 + 0.00

AN ETHNOGRAPHIC STUDY OF NIGHT BLINDNESS "*RATAUNI*" AMONG WOMEN IN THE TERAI OF NEPAL

PARUL CHRISTIAN,[1]* MARGARET E. BENTLEY,[1] RAJENDRA PRADHAN[2] and KEITH P. WEST JR.[1]

[1]Division of Human Nutrition, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD, U.S.A. and [2]NNIPS, Nepal Netra Jyoti Sangh, Kathmandu, Nepal

Abstract—Night blindness is the most common ocular condition representing moderate-to-severe vitamin A deficiency in children. Very little, however, is known about maternal night blindness, which has recently been reported to occur frequently during pregnancy in parts of south-east Asia. In Nepal, the prevalence of night blindness is reported to be 16%. We carried out an ethnographic study of night blindness during pregnancy in the south-eastern, rural plains of Nepal as preliminary research for a case-control study of the determinants of this condition. The purpose of the research was to identify local terms and concepts of night blindness and to examine women's perceptions of its causes, symptoms, severity, and consequences during pregnancy. Data collection involved in-depth interviews, case studies, unstructured observations and structured anthropologic methods, such as free listing and quick sort ranking. Women considered night blindness to be an important illness of pregnancy, ranking it second (to vaginal bleeding) in perceived severity from a list of 15 "women's illnesses". Local terms for night blindness were identified in three different languages from the region. Informants described a complex ethnomedical model of night blindness that included causes, symptomatology, and treatment alternatives. However, there was no perceived link between food intake and the occurrence of night blindness. The major causes of night blindness were attributed to pregnancy, weakness, or "hotness". Some women sought treatment for the condition but most women chose not to treat it since they believed that it was a transient condition of pregnancy. Interviews with women who had previously experienced night blindness and home-based observations of women exhibiting concurrent night blindness showed that it adversely affected their activity patterns, especially those related to child care and food preparation. Night blindness increased reliance on family members to perform various domestic chores and was also associated with personal injury and accidents. The findings of this study have relevance for women's reproductive health and nutrition throughout the Indian sub-continent. A simple history of night blindness may be a practical tool to identify women with nutritional and health risks. Maternal night blindness should be more routinely investigated in vitamin A deficient areas of the world, both to define the magnitude of the problem, and to develop programs/interventions that specifically target this population. © 1998 Elsevier Science Ltd. All rights reserved

Key words—night blindness, ethnography, pregnancy, women's work, Nepal

INTRODUCTION

Background

Scotopic vision, or the ability to see under dim illumination, is dependent on the efficient production of a photosensitive pigment, called rhodopsin, by retinal rod cells. When dim light enters the eye and falls upon the retina, it is absorbed by the rods to synthesize rhodopsin or "visual purple"—a pigment containing vitamin A (*cis*-retinal) and a protein called opsin. Once synthesized, rhodopsin responds to light energy, triggering neural impulses that are conducted along the optic nerve to the brain. Under normal conditions, synthesis of rhodopsin by the rods is rapid. Vitamin A deficiency results in delayed dark adaptation, and when sufficiently severe, leads to night blindness. Night blindness is the earliest and most common clinical manifestation of moderate-to-severe vitamin A deficiency among children of the developing world (Sommer and West, 1996). Where prevalent, night blindness is often described in local term(s) or in some cultures, even as "chicken eyes" (chickens cannot see at night as their eyes lack rod cells) (Sommer *et al.*, 1980).

Night blindness among adults is considered to be relatively uncommon, although recent evidence suggests that it may be prevalent among pregnant and, to a lesser extent, among lactating women in south-east Asia (Katz *et al.*, 1995; Bloem *et al.*, 1996; Linehan *et al.*, 1996; Malyavin *et al.*, 1996). The additional requirement for vitamin A during pregnancy is relatively small and confined mostly to the third trimester (Underwood, 1994). Maternal plasma retinol levels typically decrease during the course of pregnancy (McGanity *et al.*, 1969; Basu and Arulanantham, 1973; Jayasekera *et al.*, 1991) but in poor and under-privileged populations ha-

*Author for correspondence.

25

bitually consuming low amounts of vitamin A, this decline may reflect more than normal hemodilution of pregnancy. This could translate into acute deficiency and manifest night blindness during pregnancy.

Night blindness was first reported to occur in ancient Egypt where it was treated with topically applied liver juice or by consuming liver in the diet (Wolf, 1978). In Newfoundland and Labrador, functional night blindness was found in fishermen whose diets were markedly deficient in vitamin A (Aykroyd, 1930). Night blindness in women in this region, although less common, usually occurred during pregnancy, and when untreated, was known to disappear spontaneously after giving birth. Subsequent accounts of night blindness during pregnancy were found in the late 1960's in India from two clinic-based studies, which noted that night blindness occurred frequently among pregnant women (Dixit, 1966; Mandal *et al.*, 1969) but was not considered an important disease of pregnancy due to its transient nature (Mandal *et al.*, 1969).

Night blindness in contemporary times

Recently, in Nepal, the prevalence of night blindness during pregnancy was reported to be 16% in the south-eastern plains (Katz *et al.*, 1995). A survey conducted in the far and mid-western regions of Nepal showed that proportion of women who had ever experienced night blindness varied from 8–46%, depending on the district (Linehan *et al.*, 1996). In a previous survey from Bangladesh, 1.3–2.4% women of reproductive age reported night blindness (Bloem *et al.*, 1996), while a national survey in Lao PDR reported 11.5% of pregnant women to be night blind (Malyavin *et al.*, 1996). The survey in Bangladesh did not ascertain the pregnancy status of women but used women of reproductive age in the calculation of prevalence rate. Studies done in Mali (Dettwyler and Fishman, 1990) and India (Patel, 1994) report that the community considered night blindness to be quite common during pregnancy. In Niger, night blindness was reported to occur among women and was recognized and treated at home with food remedies (Blum and Pelto, 1996). Night blindness was negatively associated with access to water supply, ownership of household items, and head of the household literacy, but not maternal literacy (Katz *et al.*, 1995). A study done in the far-western hills of Nepal showed a moderate degree of geographic and seasonal clustering of night blindness in pregnancy, and greater frequency of the condition among low caste and older women (Starbuck, 1993).

Little is known or understood about how Nepali women perceive this condition and how important it is to them within the context of other women's illnesses. The purpose of the present study was to explore women's perceptions regarding the causes, severity, progression, and treatment of night blindness during pregnancy, and to explore the impact of night blindness on pregnant women's work and activity patterns. The ethnographic research also provided formative data for the planning and conducting of a larger, epidemiologic study of the risk factors of night blindness by identifying local terms for night blindness, and formulating hypotheses and questions on food related beliefs and behaviors of women during pregnancy. This paper explores the cultural meanings of this condition manifest during pregnancy in one setting, and demonstrates the relevance of an "emic" perspective in the development of public health programs (Pelto and Pelto, 1978).

RESEARCH SETTING

The study was conducted during September through November of 1993 in three "village development areas" in Sarlahi district, in the "*terai*" (plains) of Nepal. This area is in the south-eastern, low-lying plains, contiguous to Bihar State in India, and where 16% of women reported night blindness during pregnancy (Katz *et al.*, 1995). Its population is representative of populations from the northern Gangetic flood plains of India. Vitamin A deficiency is a problem of major public health significance in the terai of Nepal, and is more evident among preschool children (Brillaint *et al.*, 1985; Upadhyay *et al.*, 1985; Hennig *et al.*, 1991). Studies have shown ~30% reductions in child mortality after vitamin A supplementation in this region (West *et al.*, 1991; Pokhrel *et al.*, 1994). The flat plains of southern Nepal, hold more than half the population of the country. The altitude of the terai is approximately 250 feet above mean sea level. The climate is hot during the summer months (April–June), wet during the monsoon (July–September), and cool in the dry winter (October–March).

The majority of people living in the terai are called "*Madheshis*" — people who have migrated from the neighboring states of India. Other residents include a variety of ethnic groups, referred to mainly as "*Pahadis*" — those who have migrated from the hills. There are several languages and dialects spoken in the area, including Maithili, Nepali and Tharu. A majority of the people are Hindu, who are divided into four main castes and a number of sub-castes. The Hindu caste system determines, to a large extent, the social and economic status of families. A large proportion of the population living in the terai rely on agriculture as the main source of income. Land holdings are small and a significant proportion of the families subsist on wages obtained from agricultural labor. Illiteracy, especially among females, is widespread and a very small proportion of both women and men receive high school education. Sanitation and access to health care are poor. About 90% of preg-

nant women deliver at home and are assisted mostly by household members, neighbors, and relatives.

Maithili women participate in both productive and domestic activities and spend on an average more hours per day working (~10) than men (~7) (Acharya, 1981). They tend to spend less time in productive work as compared to men (40% *vs* 90%), whereas men's contribution to domestic and child care activities is marginal. Maithili women participate in planting, harvesting and weeding crops, tending to animals, collecting fodder and firewood apart from being involved in cooking, cleaning, and taking care of the children. Household economic status influences the pattern and level of women's work (Acharya, 1981; Acharya and Bennett, 1983).

METHODOLOGY

Qualitative research methods for the present research were adapted from methods developed for investigating women's health in India (Bentley *et al.*, 1992; Gittelsohn *et al.*, in press). In all, 38 interviews were conducted with pregnant women, some of whom had a current ($n = 4$) or previous history of night blindness ($n = 8$). Other participants were pregnant and lactating women ($n = 12$), traditional birth attendants ($n = 3$), women with children ($n = 6$), a local healer (1) and community health workers practicing in the study area ($n = 2$). Two currently night blind women were interviewed twice. The pregnant and lactating respondents were identified with the help of data collectors of a field study that was on-going in the same area and in some cases by networking with other informants or people in the village. For example, the traditional birth attendants or community health workers helped identify women who were pregnant or had recently delivered a baby in the community.

The interviews explored local term(s) and definitions of night blindness, and the community's description and characterization of these term(s) (Spradley, 1979). Different castes, ethnicities, and income groups were included. Although interviews were open-ended, field guides were developed for each informant type to provide structure. The field guides included questions on women's illnesses, diets, beliefs related to pregnancy and lactation, treatment seeking behaviors, the hot-cold classification system, and beliefs related to maternal night blindness. Field guides for night blindness case histories and pregnancy narratives were developed separately. Most of the interviews lasted for at least an hour, and were conducted in Maithili or Nepali with the assistance of a local translator who spoke these languages fluently. Detailed field notes were taken, and later translated and expanded into English.

Following Kleinman (1980), an ethnomedical model of maternal night blindness that describes the cultural belief system concerning the manifestation and types of, causes of, prognosis for, possible treatment for and other pertinent knowledge of a specific illness or group of illnesses was developed. The model describes each of these components based on some degree of consensus drawn from the in-depth interviews. The model has certain limitations, in that it does not represent all of the variability in women's responses because of small numbers. Instead, the model tries to capture the most commonly shared perceptions that were identified from the interviews.

Direct observations of two women currently experiencing night blindness and of one woman without night blindness were done from dusk until after dark to document how night blindness affected mealtime behaviors, food preparation and other night-time activities. Over the period of the study, only two cases of pregnant women who were currently experiencing night blindness were identified. The direct observations recorded all of the women's activities including the dynamics of assistance they received, if any, from other family members. The amount of time it took women to perform specific activities, as well as their ability to move around and work in the presence or absence of a source of light, were carefully recorded. Case studies of these two women provided a "close-up" of pregnant women with night blindness. One of the case studies was used for the construction of an individual explanatory model of night blindness (Kleinman, 1980; Gittelsohn, 1992).

Free listing (Bernard, 1994) was done to obtain a list of women's illnesses ($n = 25$) using a standard question with 2 or 3 standard probes. Houses in the three villages were visited to learn whether a married woman with one or more children lived in it. Twenty-five such households were selected and one woman from each household was interviewed. The standard question asked of women was: "Can you please tell me the names of women's illnesses?" along with standard probes such as "what else?", "are there anymore?".

Quick-sort ranking is a method used to obtain a ranking of items in a domain (subject area) for informants who can not read or write (Weller and Romney, 1988). This method uses a step-wise, paired comparison of the items that are to be ranked by a specified criterion. The same women who provided the list of women's illnesses were revisited and asked to rank 15 of the most commonly mentioned women's illnesses, in order of perceived severity ($n = 25$). The respondent was asked to pick, from two randomly selected illnesses, the one she considered more severe. This was done with all different combinations of pairs of illnesses, until a rank order of women's illnesses by severity was obtained.

RESULTS

Ethnomedical model of night blindness during pregnancy

An ethnomedical model of night blindness during pregnancy was developed using data collected from interviews of women who were currently night blind or who had experienced night blindness before (Fig. 1). Common local terms for night blindness were "*rataunî*" (Maithili), "*rataundho*" (Nepali) and "*rataunji*" (Tharu), which in all three languages literally means "night blindness" (also the biomedical term). A majority of the women used these terms, although some women described the symptoms of "poor vision at night" when referring to the condition.

Symptoms

Night blindness was defined by the informants as "no vision at night, after sunset or in the evening", "poor vision very early in the morning", "smoke in the eyes", "haziness", and "hazy vision in the light of a lamp". Several women also mentioned restriction in movement at night, accidents, or a shift in activity patterns. From the following descriptions, given by three women, it appears that night blindness is not only an absence of scotopic vision (vision in dim illumination), but may be characterized by different degrees or levels of visibility at dusk or night. This may be indicative of the variation in the degree of deficiency among this population.

"*Could not see clearly at first ... later could not see at all.*

In the early part of the evening can not see at all ... later when it gets very dark can see a little.

At first after delivery could only see hazily ... later could see clearly like before."

Causes

Virtually all of the women believed night blindness was a consequence of "pregnancy", or "having a baby in the body or in the stomach". For example a woman said "*Don't know what causes it ... it happens to some women who are pregnant, like the swelling of hand and feet. It happens on its own, because of the pregnancy*". Another reported cause was a condition called "*kamjorî*", which may be loosely translated as "weakness" that commonly manifests during vulnerable physiologic states such as pregnancy, lactation and illness and is a perceived cause of several illnesses. This condition has also been described by women in India in several ethnographic studies examining women's reproductive health problems (Gittelsohn *et al.*, 1994). A third commonly mentioned cause was associated with the Asian "hot–cold" classification system, in which the body needs to be in a state of "humoral balance" (Messer, 1989). "*Garmî*" or "hotness" re-

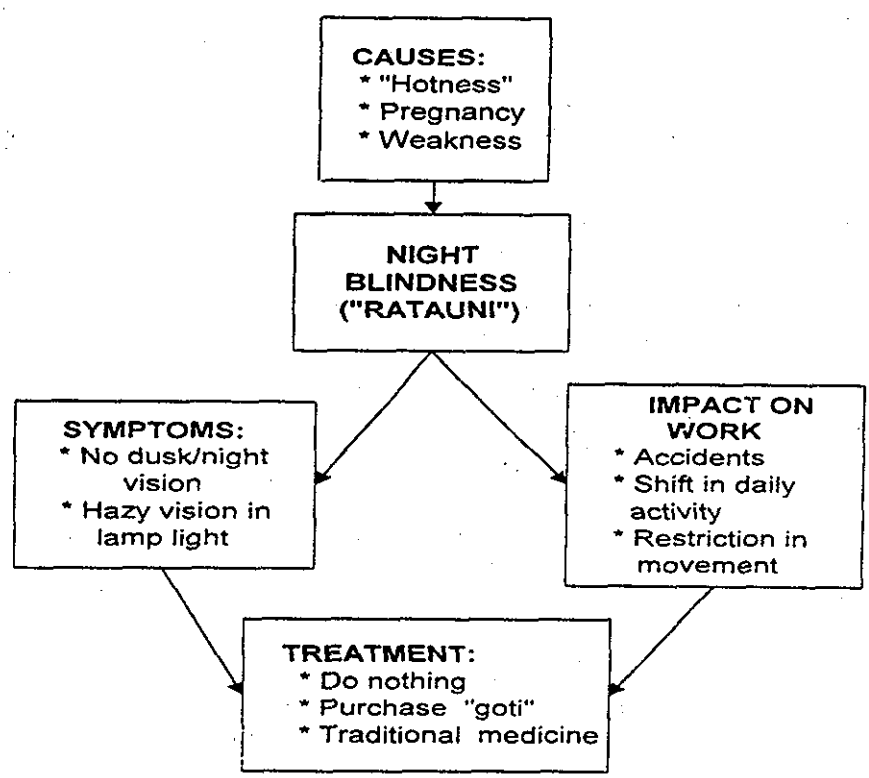


Fig. 1. Ethnomedical model of night blindness during pregnancy.

lated to the season, state of the body, or consumption of "hot" foods was also considered to be one of the causes of night blindness during pregnancy. One woman said: "*It happens when it is hot, due to the heat ... I had ratauni in Baishakh-Jeth* (May–June)".

While the night blindness was associated mostly with pregnancy, there was a general awareness that night blindness could persist into the post-partum period, or reappear during lactation. When women were asked why some pregnant women did not become night blind, respondents were unable to provide an explanation, or said that they had to be "*kamjor*" (weak) in order to become night blind.

Treatment

Treatment options for night blindness ranged from not seeking any treatment to taking vitamin A tablets, which are available quite cheaply at local pharmacies, or health posts (kiosks). There are two or three kinds of vitamin A supplements available, but the most common and popular are small, round, and red colored, containing 5,000 IU of vitamin A and 400 IU of vitamin D. These tablets are popularly known as "*ratauni goti*" or "night blindness-medicine". None of the women mentioned that these "*gotis*" contained vitamin A. The cost ranges from 3–6 gotis per one rupee (US 2 cents). Almost all women interviewed had heard about this cure for night blindness or had reported taking "*gotis*" in varied dosages as treatment for the condition. The reason why most women did not self-treat may be related to the belief that night blindness is a transient condition of pregnancy or that medicines were "hot" and should not be taken during pregnancy. For example, a woman said that, "*When the baby is born it* (night blindness) *will go away on its own ... medicine may cure it, but some people say that medicines may harm the baby*". Night blindness was perceived by many women to be part of the pregnancy experience, like morning sickness, for which usually no treatment is sought. Another reason for not treating night blindness with medicines was related to the lack of belief in its curative properties. One woman said, "*Some say it* (night blindness) *will go away with goti. For some it does not go away ... one of the women could not see even after the baby was born*". Reasons why some women self-treated night blindness and others did not may be related to previous experience with taking the "*goti*", with how debilitating the condition is, because of economic constraints, or low education level.

No woman mentioned the use of home remedies or traditional medicine. Also women did not mention diet or food in either the cause or treatment of night blindness. A few women, however, discussed night blindness in the context of food indirectly. For example, one informant mentioned eating "cold" foods to counteract the "hotness" that was perceived to cause night blindness. However, neither food nor diet was included in the general ethnomedical model of night blindness.

Progression of night blindness

Various scenarios of the onset, termination, and duration of night blindness were elicited based on 20 women's descriptions of the progression of the condition (Fig. 2). Onset of night blindness was reported to occur mostly in the third trimester or late second trimester. Most women reported that it usually disappeared quite soon after delivery, although some Maithili women were less able to tell when it went away because they are usually confined inside the house for several days after delivery. Three women reported the recurrence of night

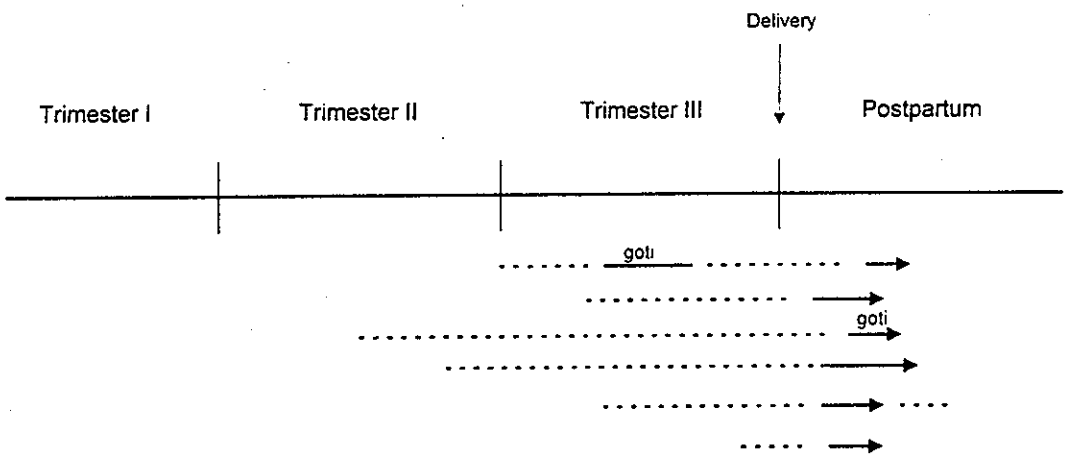


Fig. 2. Case scenarios of the progression of night blindness during the course of pregnancy. (The dashed and filled lines represent presence and absence of night blindness, respectively.).

29

blindness after delivery, and in one instance it disappeared only after the woman consumed "*gotis*" after delivery.

Saliency of night blindness

Among the first 15 most frequently mentioned illnesses, night blindness was mentioned by 24% of the women, suggesting that this condition was less salient in comparison to other conditions like headache, back ache, dizziness, stomach ache *etc.*, which were mentioned more frequently (Table 1). Although the free-listing elicited "women's illnesses", some specific conditions related to pregnancy were also mentioned. However, other general conditions experienced more commonly by women were mentioned more frequently. A quick-sort ranking exercise obtained a rank for night blindness among other women's illnesses by perceived severity. Night blindness was ranked second (to vaginal bleeding) in perceived severity among these illnesses, indicating that women did perceive night blindness to be a serious condition. One woman called it a "*bimari birah*" or "severe illness", because "*the eye is the most important thing in the world ... if you have no eyes how can you walk even a single pace in your life? ... the world would become dark*".

The ranking of illnesses by severity seemed to be inversely related to frequency with which they were mentioned by women. Commonly occurring conditions like headaches and stomach aches were mentioned more frequently, but were not perceived as very severe. On the other hand, the less common conditions, such as vaginal bleeding and night blindness, were mentioned less frequently, but were perceived to be severe by some.

Impact on work

Women both reported and were observed to work in domestic activities (food preparation, child care, cleaning and washing, cutting grass, and fetching firewood) and in productive work (agricultural labor, fishing, and small business). Evening and night-time activities of women are usually within the domestic sphere and include cooking, cleaning dishes, taking care of children and occasionally fetching water.

The impact of night blindness on women's night-time work and activity pattern was assessed by interviews and by night-time direct observations of two currently night blind pregnant women. The effect of night blindness on women ranged from total inactivity, where women just sat around or sought assistance from other members of the family, to coping by working when close to the light of a lamp or fire. The amount of time spent for child care activities and quality of care was affected; women reported relying on other family members for night-time feeding and cleaning young children. One woman said that she was not able to tend to her baby who became sick one night and

Table 1. Frequency and ranking by severity of 15 most common women'sillnesses (n = 25)

Women's Illness	Frequency *N*	Frequency %	Rank
Vaginal bleeding	13	52	1
Night blindness	6	24	2
Blood in stool	5	20	3
Kamjori	4	16	4
Parsoti	4	16	5
Menstrual problems	12	48	6
Stomach ache	25	100	7
Dizziness	25	100	8
Vomiting	10	40	9
Headache	24	96	10
Back ache	22	88	11
Hands/legs ache	25	100	12
Fever	24	96	13
Lack of appetite	10	40	14
Sleepiness	11	44	15

Kamjori and *parsoti* both represent a general feeling of weakness.

was crying. She was unable to look for any medicine for him and instead massaged him with oil in front of the fire. Night blindness also affected women's productivity in some instances. For example, one night blind woman who worked as a wage agricultural-worker said that she had to return home early from work because she could not see at dusk and getting home late was difficult. Due to this she lost a few hours of wages every day. At the time of the interview she had stopped going to work altogether. Women also described a shift in their night-time activity patterns, such as cooking dinner earlier in the day when there was light. They also reported restriction in movement, difficulty in eating the evening meal, accidents and other problems like the social stigma attached to being night blind. Several women reported falling when going to the field during early morning or late evening hours, and one woman reported bumping her head against a beam. Women who had experienced night blindness reported being teased and laughed at by neighbors and young children. The local term "*ratauni*" may also be used in a derogatory way. One woman said: "*When I was night blind small children would bother me a lot ... they would pull away my glass and plate while I was eating*". The availability of assistance from household members seemed to influence the ability of the women to cope with night blindness. One woman reported: "*I cannot see anything at night, my sisters-in-law make the rice* (food)". Some of the reported impact on activity was confirmed through the observations.

The night-time observations of two night blind pregnant women suggest a diverse impact on women's activities. One of the night blind women was observed cooking for her family, serving, and eating in the light of a lamp. There appeared to be very little impact on her ability to do activities in the light of a fire or a lamp. On the other hand, the second woman did not do anything, but sat in one place for a majority of the time during the obser-

vation. She also appeared to stare around with a vacant expression in her eyes, as though she were trying to discern objects in the dark. Although the observational data are extremely scant, they illustrate the diversity of impact night blindness may have on women's day to day lives and activity pattern.

Night blindness case studies

Two case studies of night blind women are presented below to illustrate how the causation, symptomatologies and severity of the condition can vary. The two case studies are of women from the two major ethnic groups residing in this area (Maithili and *Pahadi*). The first case study presents a detailed explanatory model of night blindness in a pregnant woman, which was due to a series of events that began during an earlier pregnancy. The second case study describes how the activity of a woman changes when night blindness is more severe.

Case study I. Gangiyadevi is a low caste Maithili woman, living with her husband and two children. She is 9 months pregnant and developed night blindness in the second trimester of her pregnancy. This is her fourth pregnancy. She has a history of early infant deaths from previous pregnancies. She has had night blindness during all the previous 3 pregnancies. She remembers that during one of these pregnancies, she developed night blindness 2–3 months before delivery, while during another pregnancy, she had become night blind just a few days before she delivered. She believes that her "*ratauni*" was caused by "*kamjori*" or "weakness". She says that the insufficient blood in her body has caused her to become weak. In the former pregnancy she had high fever and did not take any medicine because she was pregnant (women consider medicines to be generally harmful during pregnancy). She had also suffered from "wind in the knees" (*hava lagal khuttama*), and menstrual problems (*mahinbari ma gadbad*). She then had a breach birth and the baby died subsequently in 18 days. She menstruated only 2–3 times after this before she conceived again. She says that all this had resulted in her "kamjori" (weakness). Night blindness that occurred during the earlier pregnancy was cured soon after the birth of the baby. She cannot tell the exact days after the birth because she had been confined for the first six days in the delivery room. (In Maithili households, the 6 days post delivery period is considered vulnerable during which women are kept confined in the delivery room. This period is termed "soiri".) She is not taking any treatment currently for night blindness because her husband will not buy "goti" (medicine) for her. She reports that some people in the community say that if she eats "goti", her night blindness will be cured, but she has heard mixed stories about this. She hears that while night blindness in some women was cured on the same day they took the goti, in others it took longer. She knows of a woman who did not regain night vision even after her baby was born. When asked what impact night blindness had on her, she says that she has many problems because she believes that one's eyes (sight) were very important in order to do any work. She describes difficulty in moving about; her children have to lead her by the hand if she wants to go outside after dark. In the light of the lamp she can perform some activities like cooking or eating food. She describes being able to eat from her plate of rice only when a lamp is kept next to the food. She says she fell down one early morning on her way to defecate in the fields. She complains that her husband does not assist her at night when she needs help to move around, neither does he help her with cooking or serving food.

Case study II. Mailidevi is from a low-caste Pahadi household, with three children. Two of her children have died. She is 9 months pregnant and developed night blindness only 10–12 days before the interview. She lives with her husband, children, and in-laws. She found out that she was night blind one evening when she was cleaning her dishes and inadvertently threw some dirty dish-water on her two year old child who started crying. She had apparently not seen him playing nearby. She says that everyday she loses her vision in the evening when it is time to light the lamps. She can see hazily in the light of a lamp. While she can not see in the dark she can make out what is going on around her from listening to people talking. She says that her sister-in-law cooks in the evening and her mother-in-law takes care of her children. Her husband also assists her with the housework. She has not taken any medicine to cure the condition, but expects to be able to see after the baby is born. She has experienced night blindness during her first pregnancy which disappeared 2–3 days after her son's birth. She works as a laborer in the fields, but has stopped going to work since the past few days because it gets dark by the time she returns from work and she has difficulty finding her way home.

DISCUSSION

To our knowledge, this is the first study that examines night blindness within the context of women's lives and perceptions. While night blindness during pregnancy in the south-eastern terai region is reported to be high (16%) (Katz *et al.*, 1995), women from the area also perceive the condition to be quite common. Given the context of high poverty, poor diets and nutritional status and high morbidities in Nepal, night blindness poses an additional burden with which women must cope. The condition is also perceived to be a serious health problem by women. Women refer to the eye as a metaphor for light and vision, and a symbol of knowledge and perception. Clearly, a condition that

affects sight and the ability of women to work and move around is serious. However, the transient nature of night blindness and the general awareness that it disappears, in most cases, soon after delivery probably prevents women from actively seeking treatment. In some instances, women seek treatment with modern medicine, but economic and/or social constraints may prevent this.

Local terms that describe night blindness were commonly reported and this is indicative of the saliency of this condition in the community. When prevalent, night blindness is referred to by a unique word in the local language (HKI, 1992). Furthermore, night blindness was characterized as the second most severe women's illness indicating that women would probably respond to a health program targeted at preventing or resolving it. In Sierra Leone, poor women in the community considered eye infections to be the most severe or important illness (Welbourn, 1992), suggesting that ocular disease are perceived to be of importance in various communities.

The ethnomedical model showed that the attributed causes of night blindness were "hotness", pregnancy or "*kamjori*". Based on the explanatory model of night blindness of Gangiyadevi (case study 1, Fig. 3), it was evident that the perceived etiology of night blindness was very complex and involved a series of events starting in a previous pregnancy that resulted into night blindness in the current one. "*Kamjori*" or "weakness" caused by previous illnesses, losing a baby, and menstrual problems was primarily responsible for causing night blindness. The Asian hot–cold classification influences not only food selection and intake behavior but also disease causation and treatment (Messer, 1989). "*Garmi*" ("hotness") of the body, physiologic state or season was an important contributory factor in the explanatory model of night blindness during pregnancy. Hotness of the body during pregnancy was perceived to be exacerbated by the physical environment (hot season) as well as by eating "hot" foods. The same "hotness" of medicines prevented some women from treating night blindness. The occurrence of night blindness during pregnancy is the highest during the summer months of May and June, when the number of women in the last trimester (the period when night blindness is most likely to occur) also peaks (Christian *et al.*, unpublished data). Humoral imbalances are corrected by the principle of opposites; foods that are "cold" are prescribed during pregnancy, as "hot" foods exacerbate the "hotness" (Messer, 1989). Foods that were commonly considered to be "hot" were milk, meat, mangoes, papaya, hydrogenated fat (*ghee*), whereas examples of "cold" foods were yoghurt, some varieties of dark green leafy vegetables, green gourds like snake gourd and bottle gourd, pumpkin *etc.* Two women believed that night blindness could be treated with "cold" foods like green gourds or green vegetables since it was perceived to be caused

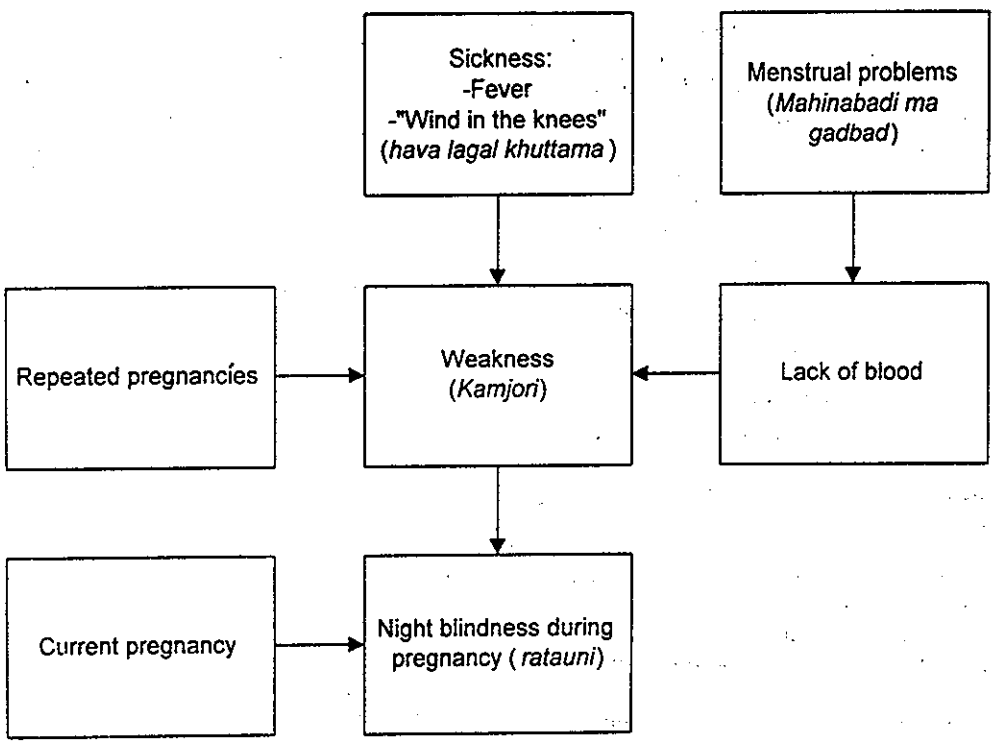


Fig. 3. Explanatory model of night blindness during pregnancy.

by "hotness". However, this belief did not seem to be commonly shared.

Although the women did not draw a direct association between food intake and development of night blindness, food related beliefs and practices of pregnancy are important to understand emerging epidemiologic associations between diet and night blindness in pregnancy. A concurrent study of dietary and behavioral risks for vitamin A deficiency among children in the same area has also shown a lack of perceived linkage between food intake and night blindness in children (Shankar *et al.*, 1997) in the presence of strong epidemiologic association between vitamin A-food intake and xerophthalmia (Pradhan *et al.*, 1994; Shankar *et al.*, 1996).

This research also identified women's use of low-dose vitamin A supplements, available in local medicine shops and pharmacies in the markets, to cure symptoms of night blindness. It was interesting that women sought "modern medicine" for the treatment of this condition, and very rarely used or mentioned using more traditional home remedies. Some women thought that treatment was pointless as the night blindness simply goes away after birth. Almost all women interviewed were aware of the treatment of night blindness with "*gotis*". The "*gotis*" were perceived to be a medicine and not a nutrient. Some interviews suggested that neighbors and friends may influence a night blind woman's decision to treat her condition. Other factors that influence this decision are willingness of the husband to assist her in this, and lack of personal access to both money to buy the gotis and the market place. This implies a reliance on the husband or other family members in seeking treatment for the condition.

Women who had experienced night blindness or were currently night blind felt that it had a debilitating effect on their ability to work. Sometimes this lead them to treat the condition with "*gotis*" despite the prevalent knowledge that it spontaneously resolved generally after delivery. Since night blindness occurs most often during pregnancy, the combined impact of pregnancy and being night blind on women's activity may be significant. There is some evidence to suggest that Tamang women of Nepal may decrease physical activity in response to pregnancy in seasons when the demand for labor is low and their contribution to household subsistence is not as essential (Panter-Brick, 1989). A study in Kenya found that "heavy" housework such as fetching water and firewood was performed less during the third trimester of pregnancy (Baksh *et al.*, 1994) suggesting that gestational age was associated with women's activity levels during pregnancy. Since night blindness occurs frequently in the third trimester its consequence for women's ability to work may be two-fold. Further, the increased level of accidents and falls among night blind women, especially if they occur in the last trimester, may potentially pose a considerable risk to the mother and her fetus.

The current ethnographic study provided a strong impetus for conducting a well-designed and executed case-control study of the risk factors of night blindness during pregnancy in the Sarlahi district of Nepal, which has revealed important risk factors associated with this condition that have significant implications for the nutritional and reproductive well-being of women (Christian, 1996). The case-control study has shown that night blind women are at a significantly higher risk of vitamin A deficiency, anemia, acute malnutrition and reproductive and infectious morbidity as compared to non-night blind pregnant women. These findings have public health implications for not only the nutritional and health status of the pregnant woman, but possibly, also for the infant.

SUMMARY AND CONCLUSIONS

The findings of this study can be generalized beyond low-land Nepal, as the people living in this region represent those living in the northern states of India like Bihar, Uttar Pradesh and West Bengal. The study found that women perceived "*rataunl*" or night blindness, which occurs quite commonly in the terai of Nepal, to be a serious condition of pregnancy which usually appeared late in pregnancy and had spontaneous remission at delivery. Despite its transient nature, women perceived it to be severe, ranking it second only to vaginal bleeding in terms of its severity, and sometimes treating it with locally available "night blindness medicine" containing vitamin A. Women attributed the cause of this condition to pregnancy, hotness of the body, food and season, or to "*kamjori*" (weakness). Women who had experienced night blindness found it to be debilitating, and to hinder their child care, food preparation and agricultural activities. The strength of the methodologic approach used for this research is that it provides an understanding of the cultural context within which night blindness occurs during pregnancy and provides an "emic" understanding of local terms for illnesses, their causes, symptomatologies and consequences. This type of research becomes crucial when trying to understand the community's perspective of a relatively new and emerging disease in a population.

RECOMMENDATIONS

Knowledge of local terms for night blindness is expected to improve the sensitivity and specificity of a history of night blindness and consequently, to identify night blindness during pregnancy as a public health problem in other parts of the developing world. Nutrition education/intervention programs would benefit from knowledge about local terms

and concepts of night blindness as this might be useful for developing messages for treatment, targeting populations, and setting up surveillance systems that identify the extent of the problem. A simple history of night blindness, a condition recognized in the community, would also be useful to include in antenatal assessments and care programs in order to target women at high risk during pregnancy who may require special nutritional support, and antenatal care and counseling. Vitamin A programs, which have been primarily focused on infants and children, need to expand to include pregnant women. Self-treatment by women who suffered from night blindness suggests that social marketing interventions for vitamin A that use the private sector and local distribution systems may be a promising strategy to adopt, both for the prevention and treatment of this condition. Programmatic research needs to be done in this area. The ultimate goal should however be to achieve overall improvement of dietary quality and vitamin A status, especially during the course of pregnancy and this should be achieved through nutrition education programs. Research is also required to conduct a more systematic investigation of the functional consequences of night blindness for the woman and her household.

Acknowledgements—This study was carried out under Cooperative Agreement DAN 0045 between the Center for Human Nutrition/Dana Center for Preventive Ophthalmology (DCPO), the Johns Hopkins School of Public Health and the Office of Health and Nutrition, US Agency for International Development, Washington, DC, and under agreement between DCPO and the National Society for the Prevention of Blindness, Kathmandu, Nepal. Additional assistance from the Task Force Sight and Life (Roche, Basel) is gratefully acknowledged.

REFERENCES

Acharya, M. (1981) The Maithili women of Sirsia. Volume II. *The Status of Women in Nepal.* Center for Economic Development and Administration, Tribhuvan University, Kathmandu, Nepal.

Acharya, M., Bennett, L. (1983) *Women and the subsistence sector. Economic participation and household decision making in Nepal.* World Bank Staff Working Papers No. 526. The World Bank, Washington, DC, U.S.A.

Aykroyd, W. R. (1930) Beriberi and other food deficiency diseases in Newfoundland and Labrador. *Journal of Hygiene* **30**, 357–386.

Baksh, M., Neumann, C. G., Paolisso, M., Trostle, R. M. Jansen, A. A. J. (1994) The influence of reproductive status on rural Kenyan women's time use. *Social Science and Medicine* **39**, 345–354.

Basu, R. J. S. and Arulanantham, R. (1973) A study of serum protein and retinol levels in pregnancy and toxemia of pregnancy in women of low socio-economic status. *Indian Journal of Medical Research* **61**, 589–595.

Bentley, M. E., Gittelsohn, J., Nag, M., Pelto, P. and Russ, J. (1992) The use of qualitative research methodologies for women's reproductive health in India. In *Rapid Assessment Methodologies for Planning and Evaluation of Health Related Programmes*, eds. N. Scrimshaw, and G. Gleason. United University Press, Tokyo, Japan.

Bernard, H. R. (1994) *Research Methods in Anthropology. Qualitative and Quantitative Approaches*, 2nd ed. Sage Publications Inc., Delhi, India.

Bloem, M. W., Huq, N., Gorstein, J., Burger, S., Kahn, T., Islam, N., Baker, S. and Davidson, F. (1996) Does the production of dark green leafy vegetables and fruits play a role in the etiology of maternal night blindness, diarrhea and malnutrition in Bangladesh. Abstract, *XVII International Vitamin A Consultative Group Meeting.* Guatemala City, Guatemala, March.

Blum, L. S. and Pelto, G. H. (1996) Beliefs and behavioral factors affecting vitamin A deficiency in Niger. Abstract, *XVII International Vitamin A Consultative Group Meeting.* Guatemala City, Guatemala, March.

Brillaint, L. B., Pokhrel, R. P., Grasset, N. C., Lepkowski, J. M., Kolstad, A., Hawks, W., Parajasegaram, R., Brilliant, G. E., Gilbert, S., Shrestha, S. R. and Kuo, J. (1985) Epidemiology of blindness in Nepal. *Bulletin of the World Health Organization* **63**, 375–386.

Christian, P. (1996) Determinants of night blindness during pregnancy in rural Nepal. Doctor in Public Health (DrPH) dissertation, School of Hygiene and Public Health. Johns Hopkins University, Baltimore, Maryland, U.S.A.

Dixit, D. T. (1966) Night blindness in third trimester of pregnancy. *Indian Journal of Medical Research* **54**, 791–795.

Dettwyler, K. A. and Fishman, C. (1990) *Field Research in Macina for Vitamin A Communications.* March 5–22, Academy for Educational Development, Washington, DC.

Gittelsohn, J., Pelto, P. J., Bentely, M. E., Bhattacharya, K. and Jensen, J. L. (in press) Rapid Assessment Procedures (RAP): Ethnographic methods to investigate women's health. International Nutrition Foundation for Developing Countries. Boston, U.S.A.

Gittelsohn, J., Bentley, M. E., Pelto, P. J., Nag, M., Pachauri, S., Harrison, A. D. and Landman, T. L. (1994) *Listening to women talk about their health. Issues and evidence from India.* Har-Anand Publications. New Delhi, India.

Gittelsohn, J. (1992) Building ethnographic models in qualitative research. *Qualitative Research Methods Newsletter*, Department of Health Services Studies, Tata Institute of Social Sciences, No. 4, New Delhi, India.

Hennig, A., Foster, A., Shrestha, S. P. and Pokhrel, R. P. (1991) Vitamin A deficiency and corneal ulceration in south-east Nepal: Implications for preventing blindness in children. *Bulletin of the World Health Organization* **69**, 235–239.

Helen Keller International (HKI) (1992) Local terms for night blindness. Vitamin A News Notes No. 8, pp. 10, 11, New York, U.S.A.

Jayasekera, J. P. D. J. S., Sunethra-Atukorala, T. M. and Senevirantne, H. R. (1991) Vitamin A status of pregnant women in five districts of Sri Lanka. *Asia-Oceania Journal of Obstetrics and Gynecology* **17**, 217–224.

Kleinman, A. (1980) *Patients and Healers in the Context of Culture.* University of California Press, Berkeley, California, U.S.A.

Katz, J., West, K. P., Khatry, S. K., Humphrey, J. H., LeClerq, S. C., Kimbrough-Pradhan, E., Pokhrel, R. P. Sommer, A. (1995) Night blindness is prevalent during pregnancy and lactation in rural Nepal. *Journal of Nutrition* **125**, 2122–2127.

Linehan, M., Shrestha, A., Ban, B. and Rycus, P. (1996) Xerophthalmia prevalence in ten districts of the far and mid-western regions of Nepal. Abstract, XVII International Vitamin A Consultative Group meeting. Guatemala City, Guatemala, March.

Malyavin, A., Beauphanny, V., Arouny, A. and Cohen, N. (1996) National vitamin A survey in Lao PDR. Abstract XVII International Vitamin A Consultative Group meeting. Guatemala City, Guatemala, March.

Mandal, G. S., Nanda, K. N. and Bose, J. (1969) Night blindness in pregnancy. *Journal of Obstetrcis and Gynecology of India* **19**, 453–458.

McGanity, W. J., Little, H. M., Fogelman, A., Jennings, L., Calhoun, E. and Dawson, E. B. (1969) Pregnancy in the adolescent. I. Preliminary summary of the health status. *American Journal of Obstetrics and Gynecology* **103**, 733–788.

Messer, E. (1989) Methods for studying determinants of food intake. In *Research Methodology in Nutritional Anthropology*, eds. G. H. Pelto, P. J. Pelto and E. Messer. The United Nations University, Tokyo, Japan.

Panter-Brick, C. (1989) Motherhood and subsistence work: the Tamang of rural Nepal. *Human Ecology* **17**, 205–225.

Patel, P. (1994) Illness beliefs and health seeking behavior of the Bhil women of Panchmahals district, Gujarat State. In *Listening to women talk about their health:Issues and evidence from India*, eds. J. Gittelsohn *et al.* Ford Foundation, Har Anand Publications, New Delhi, India.

Pelto, P. J. and Pelto, G. H. (1978) *Anthropological research. The structure of inquiry.* 2nd ed. Cambridge University Press, New York, U.S.A.

Pradhan, R., Shankar, A. V., Gittelsohn, J., West, K. P., Jr. and Dahal, B. (1994) Cultural determinants of night blindness in rural Nepal: An ethnographic study. Abst. XVI International Vitamin A Consultative Group meeting, Chiang Rai, Thailand, October.

Pokhrel, R. P., Khatry, S. K., West, K. P. Jr., Shrestha, S. R., Katz, J., Pradhan, E. K., LeClerq, S. C. and Sommer, A. (1994) Sustained reduction in child mortality with vitamin A in Nepal. *Lancet* **343**, 1368–1369.

Shankar, A. V., West, K. P. Jr., Gittelsohn, J., Katz, J. Pradhan, R. (1996) Chronic low intakes of vitamin A-rich foods in households with xerophthalmic children: a case-control study in Nepal. *American Journal of Clinical Nutrition* **64**, 242–248.

Shankar, A. V., Gittelsohn, J., Pradhan, R., Dahal, B. and West, K. P., Jr. (1997) Night blindness, diet and health in Nepalese children:An ethno-epidemiological investigation of local beliefs. *Ecology of Foods and Nutrition*, in press.

Sommer, A. and West, K. P., Jr. (1996) *Vitamin A deficiency. Health, survival and vision.* Oxford University Press, New York.

Sommer, A., Hussaini, G., Muhilal, Tarwotjo, I., Susanto, K. and Sulianti Saroso, J. (1980) History of night blindness: a simple tool for xeropthalmia screening. *American Journal of Clinical Nutrition* **33**, 887–891.

Spradley, J. P. (1979) *The Ethnographic Interview.* Holt, Rinehart and Winston Inc., Philadelphia, PA.

Starbuck, E. S. (1993) Night blindness during pregnancy as a risk marker for early infant death in Jumla, Nepal. Doctor in Public Health (DrPH) dissertation. Johns Hopkins University, Baltimore, Maryland, U.S.A.

Underwood, B. A. (1994) Maternal vitamin A status and its importance in infancy and early childhood. *American Journal of Clinical Nutrition* **59**(supplement), 517S–524S.

Upadhyay, M. P., Gurung, B. J., Pillai, K. K. and Nepal, B. P. B. (1985) Xerophthalmia in Nepalese children. *American Journal of Epidemiology* **121**, 71–77.

Welbourn, A. (1992) A note on the use of disease problem ranking with relation to socio-economic well being. An example from Sierra Leone. RRA Notes, Number 16, Special Issue on Applications for Health. IIED, London, U.K.

Weller, S. C. and Romney, K. A. (1988) *Systematic Data Collection.* Qualitative Research Methods, vol 10. Sage Publication Inc., Delhi, India.

West, K. P. Jr., Pokhrel, R. P. and Katz, J.*et al.* (1991) Efficacy of vitamin A in reducing preschool child mortality in Nepal. *Lancet* **338**, 67–71.

Wolf, G. (1978) A historical note on the mode of administration of vitamin A for the cure of night blindness. *American Journal of Clinical Nutrition* **42**, 1–24.

35

European Journal of Clinical Nutrition (1998) 52, 519–524
© 1998 Stockton Press. All rights reserved 0954–3007/98 $12.00
http://www.stockton-press.co.uk/ejcn

Working after the sun goes down: Exploring how night blindness impairs women's work activities in rural Nepal

P Christian[1], AL Thorne-Lyman[1], KP West Jr[1], ME Bentley[1], SK Khatry[2]. EK Pradhan[1], SC LeClerq[1] and SR Shrestha[1]

[1] *Center for Human Nutrition, and the Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD, USA, and* [2] *National Society for the Prevention of Blindness (Nepal Netra Jyoti Sangh), Kathmandu, Nepal*

Objective: To explore the influence of night blindness during pregnancy on nighttime work activities of women.
Design and Subjects: A community based case-control study was used to compare nighttime activities of night blind (cases) and non-night blind pregnant women (controls) using a 24 h recall method to measure work activities ($n = 116$ pairs).
Setting: Rural South-Eastern district in the plains of Nepal.
Results: Approximately one third of the night blind women reported being 'inactive' the previous night, not participating in any of the inquired work activities, as compared with only 15% of the control group ($P < 0.031$). The type of work that was significantly affected was the outdoor kind such as fetching water and washing dishes. Logistic regression analysis showed that night blind women were half as likely (odds ratio = 0.49, 95% confidence interval = 0.25 – 0.98) to work at night than women without night blindness after controlling for the effects of confounding variables including gestational age, season, and protein energy malnutrition which were significantly associated with nighttime work activity.
Conclusions: Night blindness during pregnancy, an indicator of vitamin A deficiency, reduces the number and type of work activities women perform at night, thus impairing women's ability to participate in normal subsistence activities by reducing their 'work day'.
Sponsorship: The study was funded by the Office of Health and Nutrition, US Agency for International Development, Washington DC with additional support from Task Force Sight and Life (Roche, Basel, Switzerland).
Descriptors: night blindness; pregnancy; work; activity; vitamin A deficiency; Nepal

Introduction

The daily lives of women living in rural communities of the developing world have been described as a 'zero sum game'; a situation in which women are so burdened with work that the addition of any time-consuming task detracts from their ability to perform activities necessary for the subsistence of their families (McGuire & Popkins, 1989; Bentley *et al*, 1995; Leslie, 1989). Women living in the plains of Nepal spend an estimated 10 h per day in productive, domestic and child care activities (Acharya, 1981). Even during pregnancy, women in Nepal spend the same amount of time working and expend similar amounts of energy as non-pregnant women (Panter-Brick, 1989, 1993).

This paper investigates the functional consequences of maternal night blindness, a condition caused by vitamin A deficiency, on women's lives and work activities in a rural area of Nepal. Night blindness is characterized by impaired vision at dusk and night among women who have normal vision during the daytime. In rural Nepal 10–20% of pregnant women develop night blindness during pregnancy (Katz *et al*, 1995). It is strongly associated with vitamin A deficiency and a diet that is inadequate in vitamin A-rich foods, although other co-existing risk factors such as protein energy malnutrition, anemia, and morbidity also co-exist in women suffering from night blindness (Christian *et al*, 1998a). This study probes further into this debilitating condition, caused by nutritional deficiency, and its association with nighttime work activities of pregnant women.

Methods

Research setting

The study was conducted in the Sarlahi District, located in the southern lowlands of Nepal (Terai) close to the country's border with India. The climate in the region consists of a hot season lasting from April to June, the monsoon lasting from July to September, and a cooler dry season lasting from October to March. Agriculture is the major source of income in the area, with a large proportion of the crops including rice, corn and tomatoes transported to other areas of Nepal. Female literacy is low in Nepal (14%), as is access to facilities; 18% of the population has access to adequate sanitation and 63% has safe water supply (UNICEF, 1997). None of our study population had access to electricity, an important consideration when examining nighttime activities, as the insides of houses at night are typically lit only by fires in the hearth, and

Correspondence: Dr P Christian.
Received 28 November 1997; revised 20 January 1998; accepted 27 March

36

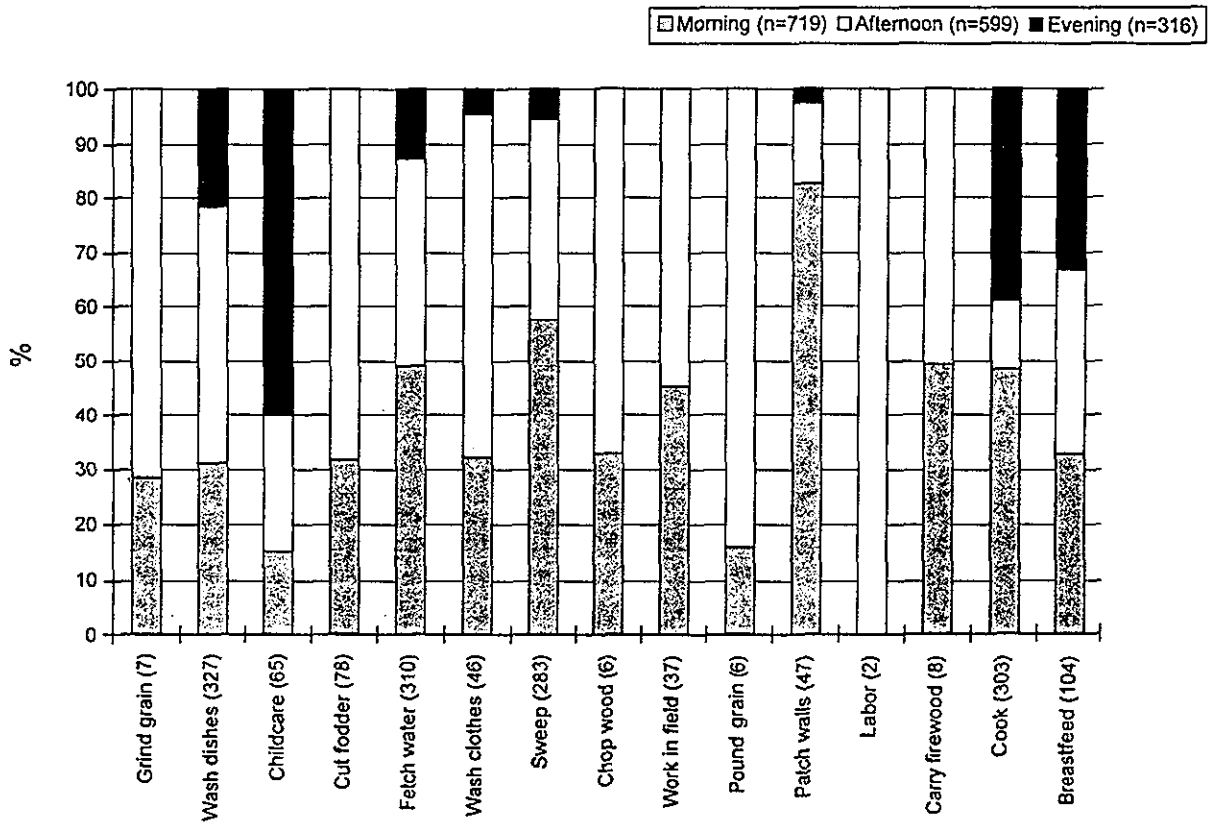


Figure 1 Proportion of work activities by general time of day reported by pregnant women in the previous 24 h. (Values shown in parentheses indicate the total number of each work activity reported by a recall of pregnant women).

Selection of the study population

Data were gathered from September, 1994 to November, 1995 as part of a population-based case-control study to examine risk factors associated with night blindness during pregnancy. The methodology is described in detail elsewhere (Christian *et al*, 1998a). A weekly pregnancy surveillance set up for a large trial of maternal vitamin A and β-carotene supplementation (West *et al*, 1997) was used to identify pregnant night blind women in the community. Local women distributing study supplements were trained to obtain weekly histories from pregnant women in their area by using local terminology for night blindness. Pregnant women reporting to be night blind were revisited by trained data collectors to verify their condition using standardized questions regarding the signs and symptoms of night blindness. Upon enrollment of each night blind case, a non-night blind control was randomly selected and assessed in the same reported month of pregnancy as the case, thus matching for month of pregnancy and seasonal variation in diet and illness. Over a period of 15 months, 116 pairs of night blind cases and matched controls were enrolled in the study. Sample size calculations were based on detecting an odds ratio of 3 or more using low serum retinol as the principal exposure.

Data collection

Cases and controls were visited within one week of their selection for an interview and an examination which included anthropometric measurements and drawing blood for estimation of hemoglobin (Hb) and serum retinol. The interview included questions about night blindness, household characteristics, previous 7 d frequency of food intake and morbidity symptoms, and a 24 h activity recall. [illegible] height, and mid upper arm circumference, were taken using standard procedures described by Gibson (1990). Hb was estimated using a portable hemoglobinometer (HemoCue Inc., Mission Viejo, California).

Assessment of work activities

Fifteen of the most frequently mentioned work activities of women from ethnographic in-depth interviews with 38 informants (Christian *et al*, 1998b) were selected for inclusion in the 24 h activity recall. Hand-drawn pictures showing women performing each of the activities were shown to the subjects while the interviewer described the activity taking place in the picture. This technique was found to be a useful approach to elicit response from a largely illiterate yet picture oriented population. Women were asked which activities they performed in three different time periods during the previous day. 'Morning' was defined as the time from waking up until eating the first of two daily meals (5 h). 'Afternoon' was defined as the time from after eating the morning meal until just before sunset (7 h), and 'night' was defined as the period from sunset until the time the woman went to bed (4–5 h). Women were then asked to sort the activities, which they reported for each time period, into two piles: one for activities that took a 'long time' and the other for activities that took a 'short time'. The decision to assess activity length in this way was made during the pre-testing of the questionnaire which showed that few women owned watches or were familiar with Western units of time estimation. From the 24 h recalls, therefore, we were able to obtain for each woman the number, type, and relative duration of each activity in which she had participated during the morning, afternoon, and night of the past day. In this paper, we focus mainly on [illegible]

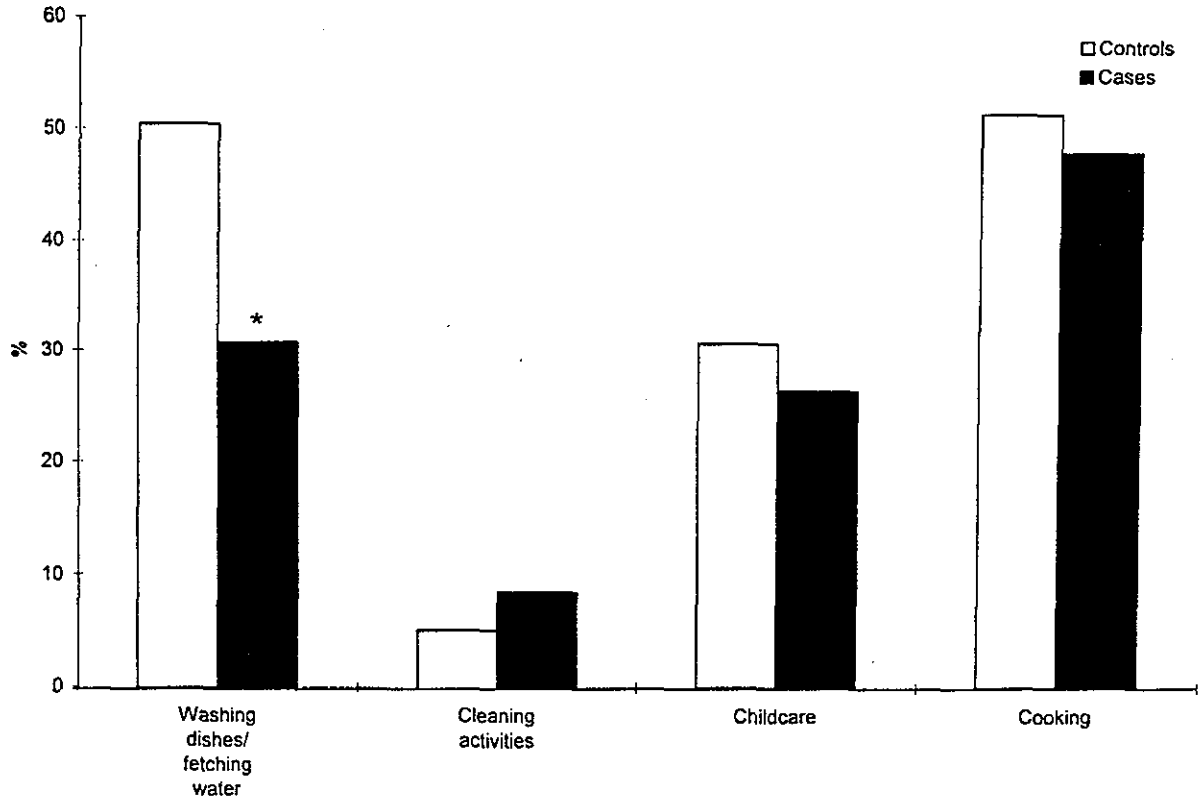


Figure 2 Participation in different types of nighttime work activities in the past 24 h by case/control status. * Significantly different ($P < 0.002$) from controls.

Table 1 Percentage of cases and controls reporting activities taking 'long' to do on the previous day

	Nighttime		*Day and nighttime*	
	Cases %	*Controls* %	*Cases* %	*Controls* %
Washing dishes	17.4	14.9	22.8[a]	13.2
Childcare	40.9	41.2	52.7	37.0
Fetching water	18.8	8.7	31.7[a]	14.5
Sweeping	22.2	0	24.8[a]	14.0
Cooking	42.9	31.7	42.7[a]	30.0
Breastfeeding	46.2	28.6	34.8	24.1

[a] Indicates cases significantly ($P < 0.05$) different from controls using chi-square test.

Table 2 Adjusted odds[a] of working at night among cases relative to *controls*

	OR (95% CI)
Night blind cases (*vs* controls)	0.49 (0.25–0.98)
Confounders:	
Hot season (*vs* cold)	0.39 (0.18–0.81)
Gestational age (weeks)	0.94 (0.89–0.99)
MUAC (cm)	1.22 (1.04–1.45)

[a] Using stepwise logistic regression. Age of the woman, parity, household size, socioeconomic status and Hb levels were not significantly associated with nighttime work activity and were excluded from the model.

Data analysis

Statistical analysis was carried out using SAS version 6.11 (SAS Institute Inc, Cary NC). The Chi-square test was used for the categorical analyses. Guttman scaling (McIver & Carmines, 1981) was used to develop a socio-economic status (SES) scale using household assets. The combination of assets that produced the best scale were ownership of land, cattle, an upper storey to the house, and a bullock cart. A five point scale ranging from 0–4 was computed by summing the four dichotomous variables (coded 0 and 1) and stratified into low and high SES groups (≤ 2 vs >2). Gestational age was calculated using data on menstrual status of women collected prospectively as part of the pregnancy surveillance of the supplementation trial, and women's report of their last menstrual period. Season was classified into hot or cold; the hot summer and monsoon months lasting from April 15 to October 15, and cold winter months for the remainder of the year. The cut-off for severe anemia was used to dichotomize women into those with Hb concentration <7 g/dl and ≥ 7 g/dl.

The purpose of the present analysis was to examine women's nighttime work activities as a consequence of night blindness (and not as an exposure) after controlling for other factors which are also known to impact work. We used multiple logistic regression analyses with nighttime activities as the dependent variable dichotomized into 0 *vs* any activity and night blindness status as the independent variable adusted for the effects of confounding variables. A stepwise logistic regression was done to select the independent variables to be included in the model. With this procedure, independent variables are entered and removed from the model in such a way that each forward selection step is followed by one or more backward elimination steps (SAS/STAT, 1990). The independent variables were case-control status, age, parity, household size, gestational age, socioeconomic status, arm circumference and Hb levels. Although this was an unmatched analysis, we adjusted for

38

the effects of gestational age and season, the two variables on which cases and controls were matched.

Ethical approval was obtained from the Nepal Health Research Council and the Johns Hopkins Committee on Human Research.

Results

Activity recall data showed that a majority of domestic indoor and outdoor activities performed by pregnant women during the previous day (cases and controls combined) were done during the daylight morning and afternoon hours (Figure 1). Some activities such as grinding grain, cutting grass for fodder, chopping and carrying firewood, and working in the fields were reported exclusively in the daytime. Other activities were also done at night on a routine basis, such as cooking (38.3%), washing dishes (21.4%), and fetching water (12.6%). A majority of reported child care activities (60%), and a large proportion of reported occurrences of breastfeeding (32%) took place during the nighttime hours.

The total number of work activities during the nighttime of the previous day showed that significantly ($P < 0.031$) more cases (31%) than control (15%) reported not doing any work at night, and conversely, fewer cases (25%) than controls (37%) participated in two or more activities at night. The influence of night blindness during pregnancy on different types of nighttime work is summarized in Figure 2. Night blind cases were less likely ($P < 0.002$) to have reported washing dishes or fetching water than controls (50% *vs* 30%). There was no difference between cases and controls reporting these two activities during the daytime (data not shown). The percentage of cases and controls who reported cleaning, cooking, and childcare and breastfeeding the previous night was not significantly different between groups.

When asked about the length of time it took to complete each activity done at night, a larger percentage of night blind women reported that the activities took a long time to do than controls, with the exception of childcare activities (Table 1). This difference was, however, not statistically significant. Combining the three time periods of the day, a significantly higher proportion of cases than controls said they took a long time to wash dishes, fetch water, sweep, and cook (all $P < 0.05$). All of these were routine activities (Figure 1) done multiple times in a day.

The influence of night blindness on the likelihood of performing nighttime activities was also examined using stepwise logistic regression analysis controlling for potential confounders (Table 2). Night blind cases were significantly less likely to do any work at night than controls (OR = 0.149, 95% CI: 0.25–0.98) after controlling for the significant effects of gestational age, season and arm circumference. A weekly increment in gestational age was associated with a 6% decrease in the likelihood of doing work at night adjusted for other factors. Nighttime work activity was seasonal; women were less likely (OR = 0.39, 95% CI: 0.18–0.81) to work at night during the hot season than in winter. MUAC was strongly associated with women's nighttime work activity. With every unit increment in MUAC, the likelihood of nighttime work as reported by women increased by 22%. Other factors such as age, parity, household size, socioeconomic status, and Hb concentration failed to be significantly associated with women's nighttime work and were excluded from the analysis.

Night blind cases and controls also differed significantly ($P = 0.039$) in the number of activities they reported during the daytime. A higher proportion of cases (40%) than controls (26%) performed a few (4 or less) activities during the day. Conversely, 47% of controls compared with 33% of cases did 5–7 activities. The percentage of women who reported doing more than 8 activities was similar between cases and controls (~27%).

Discussion

To our knowledge, this is the first study that explores the impact of vitamin A deficiency manifest as night blindness on activities of daily living or rural pregnant women. Our findings suggest that maternal night blindness impairs the ability of women to participate in normal work after the sun goes down. Women living in these environments are overworked with multiple demands on their time (Acharya, 1981). A condition like night blindness of pregnancy can reduce women's daily functional time by cutting short the productive 'work day' and affect the various roles they are expected to perform within the constraints of a few limited resources.

Our previous ethnographic study revealed that women are primarily concerned about night blindness because it limits their ability to participate in normal nighttime work activities (Christian *et al*, 1998b). Our activity data substantiate these concerns. Night blind women were significantly less likely than controls to report participation in work activities at night. Even among night blind women who did work, night blindness apparently limited their ability to do tasks which required them to go outside such as fetching water and doing dishes which both require the woman to walk some distance outside to the village water source. In contrast, cases and controls did not differ in their participation in activities which could be done by the light of the fire, such as cleaning, childcare and breastfeeding, and cooking. While other factors were associated with women's nighttime work activity, we were able to demonstrate that night blindness was independently related to doing no work at night. After controlling for the confounding effect of gestational age of pregnancy, season, and protein energy status, the odds of doing any nighttime activities by night blind women was lower by 50% in comparison to women with no night blindness.

In comparison to controls, night blind women tended to report longer times required to perform work at night. The difference was not statistically significant, perhaps because of the small proportion of women reporting doing work at night. However, cases were more likely to report taking longer to wash dishes, fetch water, sweep, and cook relative to controls when all three time periods were aggregated together. Night blind women may be spending more time during the day on work activities commonly done at night, in order to accomplish tasks which they might otherwise have to perform when lacking night vision.

Night blindness and daytime activities

Night blindness was also associated with a reduction in the number of activities reported by women during the daytime. Christian *et al* (1998a) cite numerous risk factors that cluster in night blind women in this population, including protein energy malnutrition, anemia, and reproductive illnesses. Health and nutritional factors such as anemia and protein energy malnutrition have previously

been shown to affect women's work performance (Panter-Brick, 1992; Harrington, 1983; Alemu & Lindtjorn, 1995). Nighttime work was strongly related to arm circumference measurement in the present study. Further exploration is necessary to determine the relative impact of nutritional and health factors on the activity pattern of women during the daytime.

Other factors influencing nighttime activity
Advancing gestational age of pregnancy was associated with lower nighttime work activity independent of other factors. Time allocation studies in Nepal and other developing countries, however, have shown pregnancy to have little or no effect on the total workload, or the total time women spend working (Panter-Brick, 1989, 1993; Baksh *et al*, 1994).

A likely explanation for the seasonal difference in activity lies in our operational definition of nighttime and daytime activity. Since nighttime was defined in this study as the time after sun down, longer daylight hours during the hot season probably resulted in less time to do work activities in the evening. While seasonal changes in activity (Roberts *et al*, 1982) such as in the planting and harvesting of rice have been described, it is unlikely that a one time 24 h activity recall would be able to demonstrate such differences, especially for nightime activities.

Arm circumference is an indicator of protein energy status that remains stable during pregnancy in women from less developed countries where weight gains of pregnancy are low (Krasovec & Anderson, 1991). Our results suggest that women's nutritional status as assessed by arm circumference measurements influenced women's ability to work at night. A small body size is associated with reduced ability to work (Spurr, 1990) and a low BMI in adults with low energy expenditure (Alemu & Lindtjorn, 1995). Severe anemia failed to be statistically significant as a confounder in the presence of other variables. This maybe because Hb concentration was correlated with other variables in the model such as gestational age ($r = -0.14$, $P = 0.035$), and MUAC ($r = 0.13$, $P = 0.036$). Hb is also strongly associated with case-control status (Christian *et al*, 1998a).

Limitations
Interviewers were not masked to case-control status as it was likely that they would be exposed to disease status of the informants during the course of the interview. This may have potentially resulted in an interviewer bias. Use of standardized questionnaires and adequate training and supervision was likely to minimize interviewer bias. Analysis of the daytime activities reveals that cases were doing less work even during the daylight hours which is unlikely to be due to an interviewer bias. Misclassification of case-control status maybe another potential bias which may attenuate differences between cases and controls. This type of bias was minimized by verifying night blindness histories. Furthermore, a significantly lower serum retinol concentration among cases relative to controls (Christian *et al* 1998a), was a good validation of their clinical history. Selection bias was minimal in the study as all cases of night blindness that were reported and verified to be night blind based on their symptoms were included in the study while controls were randomly picked from the population of pregnant women without night blindness.

Our data on the work activities used a 24 h recall method instead of direct observation and spot checks which are a more accurate means of exploring time allocation (Gross, 1984; Acharya & Bennett, 1982). One of the assumptions made using a recall questionnaire was that a one-time daily recall administered to a population will reflect the typical work activities and factors influencing work activities over time. The second assumption was that the total number of activities done by a woman over a period of time reflects her work load. The great diversity of work tasks done by the typical Maithili woman over the course of a day (Acharya & Bennett, 1982) supports such an assumption. However, inherent differences in the time and energy required to complete different types or work may also influence the number of activities a woman does in a day. Moreover, since we restricted our recall to the 15 most frequently performed tasks, we may have excluded other, less commonly done activities.

Conclusions

Night blindness, an indicator of vitamin A deficiency, functionally impairs women's ability to work at night independent of the potential influence of other factors, and limits the diversity of tasks women are able to perform after sunset. This pregnancy-related condition may exacerbate the constraints imposed by a pregnancy on the various social, economic, and biologic roles of women. Therefore, in addition to being an indicator of nutritional and health stress, maternal night blindness may also have adverse implications on the social and economic well being of individual women and their families in Nepal.

Acknowledgements—We thank the members of the Nepal Nutrition Intervention Project—Sarlahi for their assistance in the conduct of this study, especially DN Mandal, TR Shakiya, HG Kayastha, and Dr RP Pokhrel and to Dr J Katz and R Stallings for providing assistance with the statistical analysis.

References

Acharya M (1981): *The Maithili Women of Sirsia*. Kathmandu, Nepal: Centre for Economic Development and Administration (CEDA), Tribhuvan University.

Acharya M & Bennett L (1982): *Women and the Subsistence Sector. Economic Participation and Household Decisionmaking in Nepal*. Washington, D.C.: The World Bank.

Alemu T & Lindtjorn B (1995): Physical activity, illness, and nutritional status among adults in a rural Ethiopian community. *Int. J. Epi.* **24**, 977–983.

Baksh M, Neumann CG, Paolisso M & Trostle RM (1994): The influence of reproductive status on rural Kenyan women's time use. *Soc. Sci. Med.* **39**, 345–354.

Bentley ME, Elder J, Fukumoto M, Stallings RH, Jacoby E & Brown K (1995): Acute childhood diarrhoea and maternal time allocation in the Northern Central Sierra of Peru. *Health Pol. Plan.* **10**, 60–70.

Christian P, West KP Jr., Khatry S, Katz J, Shrestha SR, Pradhan EK, LeClerq SC & Pokhrel RP (1998a): Night blindness of pregnancy in rural Nepal—nutritional and health risks. *Int. J. Epi.* **27**, 231–237.

Christian P, Bentley ME, Pradhan R & West KP Jr (1998b): An ethnographic study of night blindness ('ratauni') among women in the Terai of Nepal. *Soc. Sci. Med.* **46**, 879–889.

Gross DR (1984): Time allocation: A tool for the study of cultural behavior. *Ann. Rev. Anthropol.* **13**, 519–558.

Gibson RS (1990): *Principles of Nutritional Assessment*. New York: Oxford University Press.

Harrington JA (1983): Nutritional stress and economic responsibility: A study of Nigerian women. In: Buvinic M, Lycette MA, McGreever WP. *Women and Poverty in the Third World*. John Hopkins University Press: Baltimore, pp. 130–156

Katz J, Khatry SK, West KP Jr., Humphrey J, LeClerq SC, Kimbrough-Pradhan E, Pokhrel RP & Sommer A (1995): Night blindness is prevalent during pregnancy and lactation in rural Nepal. *J. Nutr.* **125**, 2122–2127.

40

Krasovec K & Anderson MA (1991): Maternal nutrition and pregnancy outcome. Anthropometric assessment. Pan American Health Organization, Scientific Publication no 529. PAHO, WHO, Washington DC.

Leslie J (1989): Women's time: a factor in the use of child survival technologies? *Health Policy and Planning* **4**, 1–16.

McIver JP & Carmines EG (1981): *Unidimensional Scaling*. Beverley Hills, CA: Sage Publications.

McGuire J & Popkin BM (1989): Beating the zero-sum game: Women and nutrition in the third world. Part 1. *Food Nutr. Bull.* **11**, 38–63.

Panter-Brick C (1989): Motherhood and subsistence work: the Tamang of rural Nepal. *Hum. Ecol.* **17**, 205–228.

Panter-Brick C (1992): Women's working behavior and maternal-child health in rural Nepal. In: Norgan N (ed). *Physical Activity and Health* 34th Symposium volume of the Society for the Study of Human Biology. Cambridge University Press: Cambridge, pp. 190–206.

Panter-Brick C (1993): Seasonality of energy expenditure during pregnancy and lactation for rual Nepali women. *Am. J. Clin. Nutr.* **57**, 620–628.

Paolisso M & Leslie J (1995): Meeting the changing health needs of women in developing countries. *Soc. Sci. Med.* **40**, 55–65.

Roberts SB, Paul AA, Cole TJ & Whitehead RG (1982): Seasonal changes in activity, birthweight and lactational performance in rural Gambian women. *Trans. R. Soc. Med. Hyg.* **76**, 668–678.

SAS/STAT (1990): *User's Guide, Version 6, 4th ed vol 2*. SAS Institute Inc. Cary NC.

Spurr GB (1990): The impact of chronic undernutrition on physical work capacity and daily energy expenditure. In *Diet and Diseases in Traditional and Developing Societies*, eds. GA Harrison, JC Waterlow, pp 24–61. Cambridge: Cambridge University Press.

UNICEF (1997): *The State of the World's Children*, 1997. New York: Oxford University Press.

West KP Jr., Khatry SK, Katz J, LeClerq SC, Pradhan EK, Shrestha SR, Connor PB, Dali S, Adhikari R, Pokhrel RP & Sommer A (1997): *Impact of Weekly Supplementation of Women with Vitamin A or Betacarotene on Fetal, Infant and Maternal Mortality in Nepal.* XVIII International Vitamin A Consultative Group meeting, Cairo, Egypt. Abstract.

Pergamon

PII: S0277-9536(98)00131-2

Soc. Sci. Med. Vol. 47, No. 4, pp. 477–486, 1998
© 1998 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
0277-9536/98 $19.00 + 0.00

CHILD FEEDING AND CARE BEHAVIORS ARE ASSOCIATED WITH XEROPHTHALMIA IN RURAL NEPALESE HOUSEHOLDS

JOEL GITTELSOHN,[1]* ANITA V. SHANKAR,[1] KEITH P. WEST JR.,[1] FAISAL FARUQUE,[1] TARA GNYWALI[2] and ELIZABETH K. PRADHAN[1]

[1]Center for Human Nutrition and Division of Human Nutrition, Johns Hopkins University, School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, MD 21205-2179, U.S.A. and [2]Nepal Nutrition Intervention Project-Sarlahi, Nepal Eye Hospital, Kathmandu, Nepal

Abstract—The study examined caregiver–child interactions, intrahousehold food allocation and general child care behaviors and their effect on children's xerophthalmia status in the rural Terai region of Nepal. Seventy-eight households with a child having a history of xerophthalmia (cases) were matched with 78 households with a child of the same age having no history of xerophthalmia (controls). Seven day-long continuous monitoring observations were performed in each household (over 15 months) by trained Nepali observers, focusing on feeding and care of a focus child and his/her younger sibling. Nineteen different behavioral variables were operationalized, including serving method, second helpings, serving refusals, encouragement to eat, request intensity, meal serving order, food channeling, food sharing, positive social behaviors, negative social behaviors, and positive health behaviors. Automatic serving and request intensity were strongly negatively correlated, especially among younger siblings. Children who serve themselves receive less encouragement to eat. Those children who are refused in their requests for food tend to ask for food more frequently, for a longer time, and be less likely to self-serve. Children who eat from a shared plate are less likely to interact with a food server and more likely to self-serve. Negative social behavior towards children is associated with the child having to request food more frequently and a greater likelihood of being refused food. Children who receive positive health care from their caregivers are also more frequently asked if they would like food by the server and are encouraged to eat. Several caregiver–child feeding behaviors were related to a child's risk of having past vitamin A deficiency. Controls were much more likely to be served food automatically. Cases were more likely to serve themselves food and have multiple servings of food. Cases were nearly two times more likely than controls to be treated with neglect or harshly, and much less likely than controls to have their health needs receive attention. Examining intrahousehold behavior is critical for understanding the causes of vitamin A deficiency in rural Nepalese children, and has great potential for identifying and improving interventions to improve children's diets and care. © 1998 Elsevier Science Ltd. All rights reserved

Key words—child feeding, child care, household behaviors, xerophthalmia, Nepal

INTRODUCTION

The influence of caregiver behavior on child food intake varies in different settings, ranging from a high level of food consumption promotion exhibited by the caregiver, to relatively low levels of promotion (Dettwyler, 1989). In some settings, caregivers actively promote child food consumption, particularly in response to clues from the child. In Nigeria, force-feeding of children is common (Meldrum, 1984). Bentley *et al.* (1991) found that caregivers in Peru tend to encourage their children to eat when they are ill. In Nicaragua, Engle and Zeitlin (1996) found that mothers were likely to actively feed their child when the child expressed disinterest. Evidence in other settings indicates that caregivers may do relatively little to promote food consumption in children (Ashby, 1985; Dettwyler, 1986).

Much of this recent work has used direct observation methods, primarily continuous monitoring techniques. Direct observation of behavior, when feasible, is generally regarded as being more accurate than recall in obtaining behavioral information (Bentley *et al.*, 1994) as people tend to report performing positive health behaviors more frequently than they are actually observed (Stanton and Clemens, 1987; Curtis *et al.*, 1993).

While the literature is now fairly well developed in terms of describing caregiver–child interaction around feeding (see Dettwyler, 1989; Bentley *et al.*, 1995; Engle and Zeitlin, 1996), several large gaps remain. There has been little attempt to link feeding behavior directed towards a specific child to the feeding of other household members. In most settings, child feeding takes place within a household context, which greatly shapes and influences how a

*Author for correspondence.

42

particular child may be fed. Patterns of food allocation, gender and age, as well as individual preferences for foods can also affect the caregiver–child feeding interaction.

In addition, relatively little work has been done describing the relationship between child feeding and other aspects of child care within the household. Do mothers who feed their children well also care better for their children in other ways? For example, does a mother who encourages her child to eat more also show more affection or more frequently bathes her child, or is there a trade-off? Is time spent interacting with the child during mealtimes at the expense of interaction at other times? Finally, what are the health implications (if any) for caregiver–child feeding and other child care behaviors?

Figure 1 presents a model emphasizing these unexplored relationships. The caregiver (frequently the food server) mediates between the food supply and service to a particular ("focal") child and to other household members, particularly during meals. However, all household members, except for the very youngest children, can also draw on that food supply directly, perhaps with little or no input from the caregiver, especially during snacks. In addition, a focal child and other household members may exchange and share foods. All these different food-related behaviors are part of a larger set of child care behaviors that occur within the household that may influence the health status of the individual child.

This paper seeks to test this model by looking at the care and feeding of children in rural Nepal. We suspect that caregiver–child behaviors directly influence and are influenced by how food is allocated within the household, and how the child is cared for in other ways. We also feel that these behavioral patterns play a direct role in the health status of the child, but the strength of these associations is not known. To test these hypotheses, a series of continuous monitoring observations were used to assess the care and feeding of young Nepali children with and without xerophthalmia, an ophthalmic disease due to chronic, underlying dietary vitamin A inadequacy (Sommer and West, 1996).

METHODS

Study design

This case-control study was conducted in three village development committees (VDCs), totaling ~15,000 people in Sarlahi District, located in the central lowland region of Nepal. Children with clinical signs of xerophthalmia or having a current history of night blindness were identified during a community-based screening by senior ophthalmic assistants employing World Health Organization criteria (Sommer, 1982). Eighty-six households were identified as having at least one xerophthalmic ("focus") child between the ages of 1 to 6 years. Cases were treated with 200,000 IU of vitamin A on two consecutive days and referred to the local health post for follow-up. The pool of control households included those whose surveyed children exhibited no xerophthalmia and who reported no history of night blindness for any sibling or the child's mother. Control and case households were matched based on the age of case and control focus children (±3 months), and the age of the next younger siblings (±4 months), if present in the case household. Five cases either declined to participate or migrated from the region, and an additional three case households were censored due to migration of their control counterpart, resulting in 78 case-control pairs included in the analysis. Some

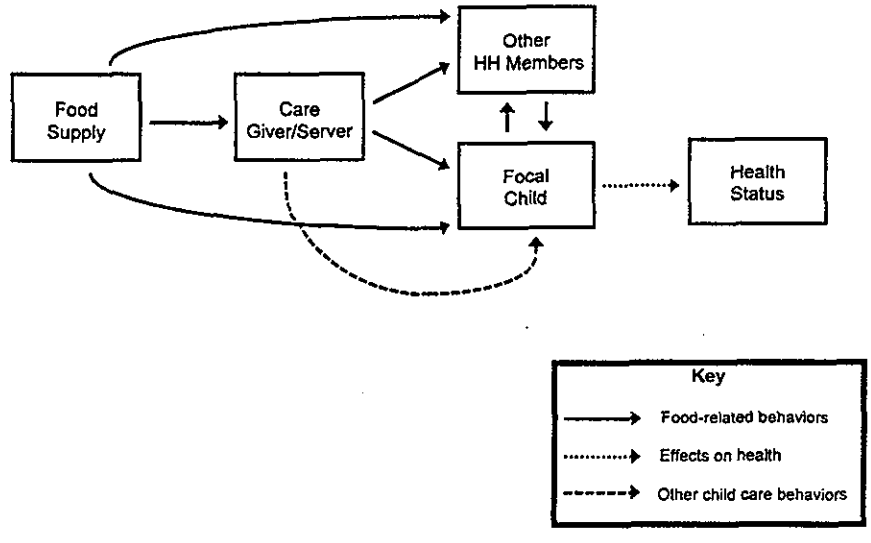


Fig. 1. Model of the relationship between caregiver–child behaviors and other household members

analyses were performed in relation to the younger siblings of cases and controls. In these instances, only 53 case and control households were included in the analyses.

Continuous monitoring observations

Seven day-long observations were conducted in each of the study households during the period from March 1993 to June 1994. Observations in a household were spaced roughly two months apart on randomly selected days of the week. Ten male local high school graduates were trained for three months as observers in portion size estimation and were standardized in the use of the behavior recording instrument (Gittelsohn *et al.*, 1994). Selection of key behaviors for recording was based on an initial six-month period of ethnographic fieldwork in the study communities.

Observations were event-focused; each time a behavior of interest occurred, it was recorded. A minimum of one record per five minutes was required when behaviors remained constant for prolonged intervals, e.g. playing, walking, sleeping. Priority was given to observing the behavior of a focal child 1–6 years old, his or her next youngest sibling (if any), and that of caregivers for those children. However, observations were recorded for other household members, particularly other children. During meals and snacks, the behavior of all household members present was monitored. Observers recorded the following information regarding each behavioral event: time, location of behavior, actor doing behavior, recipient of behavior (if any), actual behavior (or activity) observed, food name (if any), food condition, and food quantity. Observations were conducted from early morning (6–7 a.m.) until the focal child went to sleep for the night and the evening meal had been consumed (usually around 8 p.m.). The observer took a 3–4 h break in the middle of the day.

Observed behaviors were recorded as 5 letter mnemonic codes, each representing a Nepali phrase. In total, 110 different behavioral codes were developed for the study. The codes covered seven topics: child care, food preparation and serving, general, observer-related, personal hygiene and health, conversation, and work. In total, 1103 days of observation were collected.

Controlling reactivity

Because of the intensive nature of the observations, efforts were made to reduce reactivity on the part of study household members. Observations were conducted only after the household had been contacted on several occasions and was thoroughly familiar with study personnel. Observers were assigned to 20 households (10 case households and their 10 matched controls) which they consistently observed from the beginning of the study until its end. We felt that this system of assignment of study households to observers served to reduce reactivity, as there were no new faces after the first observation. In this way, systematic bias due to the presence of a particular observer was also controlled for in the comparison of case and control households. In addition, observers were blinded to case or control status of each household.

Defining behavioral counts, scales and scores

In order to summarize the continuous monitoring observation to make comparisons and identify patterns, three types of variables were constructed: counts, scales and scores.

Counts refers to the simple summing up of the number of times a single behavior occurs (or sometimes, dichotomized into whether or not a behavior occurs) over a specific time period. For instance, counting the number of handwashings, whether or not soap is used, etc. *Scales* refers to combining different behaviors according to an underlying construct. For example, scales of encouragement to eat have been developed for Peru (Bentley *et al.*, 1994) and of child demand for food in Nicaragua (Engle and Zeitlin, 1996). Items in a scale may be simply added together or weighted and then summed. *Scores* refer to a more complex form of counting, where the results of two or more behaviors, or of a behavior and other sources of information are combined. One example is the amount of time between a request for food and its actual service to a child. As another example, the principal author's work in Nepal (Gittelsohn, 1989, 1991) developed a channeling score that took into account the number of different foods consumed by an individual (the sum of many eating behaviors for that individual) and divided that by the total number of different foods consumed in the household that day (the sum of many eating behaviors for all household members).

Generally, values for each variable are calculated at the level of the observational visit, summed, and then averaged for all visits to the household. Scales are additive and, where indicated, Cronbach's α has been calculated as a measure of reliability (Cronbach, 1951). As a general rule, an α of 0.60–0.65 is considered low, 0.65–0.70 is acceptable, 0.70–0.80 is respectable, and 0.80–0.90 is good (DeVellis, 1991). For the most part, the reliability of our scales are lower than desired, suggesting at first that these scales may be measuring behaviors that are unrelated. On the other hand, earlier correlation analyses indicated significant associations between variables used in scales. We feel that the very low frequency of many of the behaviors noted during the observations contributed to the scales' low internal consistency.

Some of the created variables require differentiating between snacks and meals. These differences were described by considering the number of different foods served to the child, amount of food served to the child, and number of people present

44

during the food consumption episode (Table 1). In general, snacks are assumed to be eating events involving fewer foods in smaller amounts, and attended by fewer people than meals.

Behavioral variables used in this study

The derivation of four of the variables, serving method, meal serving order, channeling, and second helpings, has been described previously, and are among scores developed by the first author in his earlier study of intrahousehold food allocation in Nepal (Gittelsohn, 1991). The remainder of the variables have been developed from this data set, and have been divided into caregiver–child behaviors, intrahousehold food behaviors and other child care behaviors in the description below.

Caregiver–child behaviors. Eight different behavioral variables were operationalized to describe aspects of caregiver child interactions:

Serving method: actually four related scores which describe the proportion of food service behaviors during meals and snacks, where the study child is the recipient, according to four different styles: serving without asking (i.e. automatic serving) (AS), server asks then serves (SA), recipient (child) asks (RA) and self-service (SS) by the child. The four scores total 100% for any individual. Breast-feeding episodes are not included in the calculation of this score.

Second helpings: a score which describes the proportion of all the different foods offered to or served to the child more than once during observed meals and snacks.

Serving refusals: a weighted additive score combining three different types of refusals to serve a child requesting food during meals and snacks. Refusals were coded as non-discriminatory (weighted 1), ignoring the child's request (weighted 2) and discriminatory (weighted 3).

Encouragement to eat: an additive scale ($\alpha = 0.28$) combining 11 different food serving/offering behaviors to the child (e.g. automatically serving the child, asking if the child is hungry, hand feeding, etc.) during meals and snacks. Values reflect the number of times during the observation day that the child was encouraged to eat by the food server, regardless of whether or not the child actually received or ate food.

Request-service gap: a score which measures the average amount of time (minutes) between a child's request for food and actually receiving or being served food during meals and snacks.

Request intensity: a score which sums the number of times food is requested by a child before it is served during meals and snacks.

Snacking frequency: a count which refers to the number of non-meal episodes per observation day at which the child receives some food.

Meal frequency: a count which refers to the number of meal episodes per observation day at which the child receives some food.

Intrahousehold food behaviors. Five variables were operationalized to describe intrahousehold behaviors:

Meal serving order reflects relative serving order of the child during meals only. Scores range from 0 to 1, with numbers closer to 1 indicating higher serving order priority.

Channeling: a score which describes the proportion of all the different foods served to the child in the household during meals and snacks. Scores range from 0 to 1, with numbers closer to 1 indicating that the child received more of the different foods consumed in the household that day. Breastmilk was not included as a food in the calculation of this score.

Shared plate: a score which sums the number of times a child eats food off the same plate as another household member (adult or child) during meals and snacks. An observation day was divided into two sections; the morning observation period and the evening observation period. If a child ate off a shared plate during either of those two observation periods, he received a score of one for that day. If he ate off a shared plate during both those periods he received a score or two. Values range from 0 to 14.

Inter-plate food sharing: a score which measures how frequently the child receives or is offered food from another household members' own served food during meals and snacks. This variable does not include shared plate eating, where two or more individuals eat from the same plate.

Meal attendance: a count of the number of people present during a meal where a child was served.

Other child care behaviors. Four variables were operationalized to describe other aspects of child care behavior. Based on our ethnographic work in this setting, a series of socially positive and socially negative behaviors were identified when directed at a child.

Positive social behaviors is an additive scale ($\alpha = 0.56$) in which the child is the recipient of behaviors felt to be beneficial by local standards.

Table 1. Definition of snacks vs. meals for children

	1 food		2+ foods	
Amount of food served to child	≤2 people	3+ people	≤2 people	3+ people
≤50 g first serving)	snack	snack	snack	meal
> 150 g first serving)	snack	meal	meal	meal

45

The scale includes instances of affectionate gestures, holding the child, massaging the child and putting the child to sleep.

Negative social behaviors is an weighted additive scale ($\alpha = 0.39$) which includes four different types of negative treatment of the child, ranging from scolding (weighted 1), to mild slapping (weighted 2), to moderate hitting (weighted 3), to severe beating (weighted 4). This scale may also be described as a punishment or discipline scale.

Positive health behaviors is an additive scale ($\alpha = 0.56$) which includes picking lice, brushing teeth, cleaning up feces in a sanitary manner, and using soap for cleaning/washing, where the recipient of the action is the child.

A scale of negative health behaviors was also created but was removed from the analysis due to very low internal consistency of the score.

Socioeconomic status (SES) was estimated in this population through an interview with the male head of each household, and was based on ownership of eight items (flashlights, watch, bicycle, clothes cupboard, beds, number of rooms in house, roof, and pumpset). The SES scale has an acceptable Cronbach's α of 0.78. An estimate of level of reactivity of the observation has been calculated for each observational visit and is included in selected analyses (see Gittelsohn *et al.*, 1997, for details in creating this score).

Pearson's correlation coefficients, controlling for age of child, were calculated for behavioral counts, scales and scores. Coefficients having a $<5\%$ probability of a type 1 error were considered significant. Matched-pair odds ratios (OR) with 95% confidence intervals (CI) were calculated by conditional logistic regression to estimate the risk of a case not performing a behavior or performing a behavior less often than a control. To improve the meaningfulness of the parameter estimates, most scales and scores were broken down into quartiles (Q1, Q2, Q3, Q4). The first quartile (Q1) or lowest grouping was not included in the regression. Therefore, all odds ratios should be interpreted in relation to the first quartile. An exception is the meal attendance count which was dichotomized at the mean. Odds ratios are adjusted for SES and the reactivity score. Statistical analysis was carried out by using SAS version 6.1 (SAS Institute Inc, Cary, NC).

Verbal informed consent was obtained from families prior to their participation. This study was approved by the Nepal Health Research Council, Kathmandu, Nepal and the Committee on Human Research, Johns Hopkins School of Hygiene and Public Health, Baltimore, USA.

RESULTS

At the beginning of the study, the age of the focus child ranged from 1 to 6 years, with a mean age of 4.2 years. The focus child had a younger sibling in 68% of the study households, with an age range of 2 months to 4 years (mean of 1.7 years).

Caregiver–child feeding behavior

Table 2 presents statistically significant correlations between the caregiver–child feeding variables for focus children and younger siblings in case and control groups combined. Automatic (AS) and request intensity were strongly negatively correlated, especially among younger siblings ($r = -0.69$). The server asking is strongly positively correlated with encouragement to eat ($r = 0.84$ and $r = 0.63$). Younger siblings who are automatically served tend to have fewer serving refusals, and a shorter gap between request and service of food. Children who serve themselves receive less encouragement to eat, and also experience fewer refusals to their requests for food. Those children who are refused in their requests for food tend to ask for food more intensely ($r = 0.72$ and 0.56) and for a longer time, and be less likely to self-serve.

Caregiver–child feeding in relation to other household members

Table 3 presents correlations between the caregiver–child food serving and intrahousehold food behaviors. Meal serving order appears to be negatively correlated with the server asking the focus child to eat and with encouragement to eat of this child. On the other hand, channeling of foods consistently correlates with caregiver–child behavior. For both the focus child and the younger sibling, channeling of food relates to positive caregiver behaviors such as offering second helpings, encouraging the child to eat and providing frequent snacks. Channeling appears to be particularly linked to the behavior of younger siblings, such as the number of requests and frequency of serving refusals. Channeling of foods to the younger sibling is associated with the server asking if the child would like a particular food, but is negatively correlated with being served automatically and the child serving himself.

Children who eat from a shared plate are less likely to interact with a food server ($r = -0.26$ and -0.24), and more likely to self-serve ($r = 0.41$ and 0.50). These children are also less likely to be encouraged to eat by a caregiver. Interplate food sharing (the allocation of food from an individual's served portion to the child) relates more strongly to feeding behavior of the focus child than the younger sibling. Inter-plate food sharing is associated with second helpings and with refusals for food service, indicating that the child's requests for food cannot be met from the existing food supply, and must be met from served food on the part of another household member. Interplate food sharing directed to the focus child is associated with frequent requests for food, and a decrease in self-serving of food.

46

Table 2. Correlations between caregiver–child behaviors for focus child and younger sibling[1,2]

Caregiver–child food serving behaviors	Serving method: Automatic	Serving method: Server asks	Serving method: Recipient asks	Serving method: Self-service	Second helpings	Serving refusals	Encouragement to eat	Request-service gap	Request intensity	Snacking frequency
Serving method										
Server asks	0.05[3]	×								
	−0.47*[4]									
Recipient asks	−0.33*	0.17*	×							
	−0.72*	0.31*								
Self-service	−0.42*	−0.60*	−0.58*	×						
	−0.24*	−0.32*	−0.38*							
Second helpings	−0.20*	−0.09	0.19*	0.05	×					
	0.12	0.01	0.09	−0.28*						
Serving refusals	−0.11	0.01	0.41*	−0.22*	0.24*	×				
	−0.20*	0.28*	0.36*	−0.32*	0.38*					
Encouragement to eat	−0.00	0.84*	0.23*	−0.59*	0.13	0.23*	×			
	−0.13	0.63*	0.18	−0.42*	0.21*	0.33*				
Request-service gap	−0.11	−0.02	−0.01	0.10	0.07	0.29*	−0.00	×		
	−0.22*	−0.00	0.11	0.18	−0.05	0.20*	−0.08			
Request intensity	−0.26*	0.03	0.70*	−0.34*	0.10	0.72*	0.15	0.18*	×	
	−0.69*	0.36*	0.88*	−0.32*	0.10	0.56*	0.19*	0.30*		
Snacking frequency	−0.01	−0.14	−0.01	0.08	0.16*	0.18*	0.08	0.05	−0.00	×
	−0.33*	0.09	0.25*	0.10	−0.09	0.11	0.18	−0.14	0.14	
Meal frequency	−0.09	0.17*	0.05	−0.06	0.08	0.04	0.19*	−0.08	0.03	−0.29*
	0.08	0.04	0.14	−0.31*	0.54*	0.27*	0.12	−0.01	0.15	−0.20*

*$p < 0.05$ or less.
[1]Partial Pearson's correlations, controlling for age of child.
[2]$n = 156$ focus child households, $n = 106$ households with younger siblings.
[3]Correlation for focus children.
[4]Correlation for younger siblings.

Table 3. Correlations between caregiver–child feeding and intrahousehold feeding variables for focus child and younger sibling[1,2]

Caregiver–child food serving behaviors	Intrahousehold food behaviors				
	Meal serving order	Channeling	Shared plate	Inter-plate food sharing	Meal size
Serving method					
Automatic	0.08[3]	0.00	−0.06	−0.00	−0.09
	−0.02[4]	−0.33*	−0.03	0.00	0.02
Server asks	−0.30*	0.10	−0.26*	−0.01	−0.19*
	−0.04	0.41*	−0.24*	−0.13	0.03
Recipient asks	0.10	0.07	−0.33*	0.31*	0.14
	0.12	0.38*	−0.27*	0.13	0.01
Self-service	0.13	−0.10	0.41*	−0.21*	0.06
	−0.09	−0.24*	0.50*	−0.08	−0.05
Second helpings	0.09	0.32*	0.04	0.23*	0.04
	0.11	0.24*	0.07	0.24*	−0.09
Serving refusals	0.00	0.09	0.12	0.34*	0.05
	−0.02	0.29*	−0.15	0.22*	0.00
Encouragement to eat	−0.28*	0.30*	−0.20*	0.14	−0.22*
	−0.12	0.44*	−0.20*	0.01	−0.06
Request-service gap	−0.02	−0.01	0.04	0.21	0.04
	0.00	0.03	0.02	−0.03	0.09
Request intensity	0.01	0.01	−0.14	0.30*	0.13
	0.03	0.35*	−0.29*	0.05	−0.04
Snacking frequency	0.02	0.42*	0.12	0.19*	−0.17*
	−0.08	0.38*	0.01	ns	−0.08
Meal frequency	−0.14	0.14	0.03	−0.08	−0.23*
	0.00	0.28*	0.18	0.19	−0.23*

*$p < 0.05$ or less.
[1]Partial Pearson's correlations, controlling for age of child.
[2]n = 156 focus child households, n = 106 households with younger siblings.
[3]Correlation for focus children.
[4]Correlation for younger siblings.

Meal attendance is uncorrelated with most caregiver–child food serving behaviors. In larger meals gatherings, it appears that the focus child tends to be neglected, receiving fewer offerings of food, and less encouragement to eat.

Child care and child feeding

Table 4 presents relationships between caregiver–child feeding and child care behaviors. Positive social behavior is associated with automatic serving and is negatively associated with self-serving of both the focus child (r = 0.36 and −0.28, respectively) and their younger sibling (r = 0.47 and −0.27). Positive social behaviors are more often correlated with ways in which younger siblings are fed. Younger siblings who receive a lot of positive social treatment have to request food less frequently and are more likely to receive second helpings. Focus children receiving positive social behaviors are also more likely to be encouraged to eat.

Caregivers acting in a negative way toward children is associated with the child having to request food more frequently (r = 0.36 and 0.23) and a greater likelihood of being refused food (r = 0.45 and 0.28). Interestingly, negative treatment of the child is also associated with more frequent second helpings and greater encouragement to eat. Perhaps the child is not served sufficient amounts of food initially, forcing further requests for food.

Children who receive positive health care from their caregivers are also more frequently asked if they would like food by the server and are encouraged to eat. Such positive treatment is also associated with the focus child being automatically served, and not having to self serve.

Feeding and other behaviors and risk of vitamin a deficiency

Table 5 presents the results of a series of conditional logistic regressions with past xerophthalmia status of the focus child as the dependent variable. Odds ratios have been calculated to express the risk associated with behavioral variables for past xerophthalmia. The odds ratios are presented adjusted by SES of the household and reactivity score.

Several caregiver–child feeding behaviors were related to a child's risk of being chronically vitamin A deficient, reflected by a previous history of xerophthalmia (Table 5). Automatic serving of food is associated with a 83% (Q4) or 73% (Q3) reduction in risk for xerophthalmia in the children, compared to the lowest quartile. High scores (Q4) for self-service and second helpings of food are associated with a four times and eight times greater risk for xerophthalmia, respectively. None of the other scales and scores for caregiver–child feeding behaviors showed significant differences between cases and controls.

High meal attendance was associated with a 52% reduction in risk for xerophthalmia in children. While none of the other scales for intrahousehold food allocation behaviors were significant predictors of xerophthalmia status at the 95% confidence level, both channeling and inter-plate food sharing approached significance. Cases tended to be the

48

Table 4. Correlations between caregiver–child feeding and child care variables for focus child and younger sibling[1,2]

Caregiver–child food serving behaviors	Child care behaviors: Positive social	Negative social	Positive health
Serving method			
Automatic	0.36*[3]	−0.13	0.33*
	0.47*[4]	−0.20*	−0.07
Server asks	0.08	0.08	0.29*
	−0.17	0.14	0.38*
Recipient asks	−0.01	0.21*	−0.05
	−0.23*	0.20*	0.01
Self-service	−0.28*	−0.11	−0.34*
	−0.27*	−0.05	−0.14
Second helpings	0.02	0.22*	−0.06
	0.31*	0.20*	0.04
Serving refusals	0.03	0.45*	−0.05
	0.04	0.28*	0.23*
Encouragement to eat	0.25*	0.28*	0.32*
	−0.05	0.24*	0.38*
Request-service gap	−0.04	0.01	−0.13
	−0.16	−0.03	−0.05
Request intensity	−0.02	0.36*	−0.10
	−0.26*	0.23*	0.13
Snacking frequency	0.02	0.21*	0.06
	−0.02	0.18	0.04
Meal attendance	0.16	0.06	0.12
	0.25*	0.09	0.22*

*$p < 0.05$ or less.
[1]Partial Pearson's correlations, controlling for age of child.
[2]n = 156 focus child households, n = 10 households with younger siblings.
[3]Correlation for focus children.
[4]Correlation for younger siblings.

recipients of channeled foods and be involved in inter-plate food sharing.

With respect to non-feeding interactions in the households, cases were more than twice as likely to be treated with neglect or harshly, reflected by the negative social behavior scale (Q3). On the other hand, very high (Q4) positive health behavior scale scores were associated with a 66% reduction in risk for xerophthalmia.

DISCUSSION

The findings presented here underscore the importance of the household behavioral environment in producing a healthy child (here specified as non-xerophthalmic). We were able to develop a series of behavioral scales and scores that describe different aspects of the caregiver–child relationship, both food-related and directed to other aspects of child care, and relate these to intrahousehold food allocation patterns of high risk and lower risk children with respect to vitamin A deficiency. All three categories of behavior, caregiver–child, intrahousehold and child care, appeared to exert some influence on children's health. However, caregiver–child and other child care behaviors appeared to be more important than intrahousehold food allocative behaviors in determining a rural Nepali child's risk for xerophthalmia.

The tendency for cases to receive food from another's already served food may reflect receipt of relatively small initial servings of foods that require the focus child to request food from others, also supported by the higher frequency of second helpings in cases. It is interesting that large meal gatherings were protective in their association with

Table 5. Effect of caregiver child feeding, intrahousehold feeding and child care behaviors on risk of vitamin A deficiency of focus child

Behavioral variables		Odds ratio[3] (CI) (adj. SES, reactivity)
Caregiver–child feeding behaviors		
Serving method		
Automatic	Q2	0.49 (0.18–1.35)
	Q3	**0.27 (0.08–0.90)**
	Q4	**0.17 (0.04–0.70)**
Server asks	Q2	0.97 (0.40–2.31)
	Q3	1.20 (0.46–3.12)
	Q4	1.00 (0.34–2.89)
Recipient asks	Q2	1.25 (0.45–3.50)
	Q3	0.43 (0.14–1.35)
	Q4	0.92 (0.30–2.83)
Self-service	Q2	1.89 (0.71–5.06)
	Q3	2.13 (0.83–5.45)
	Q4	**4.04 (1.00–16.35)**
Second helping	Q2	2.80 (0.95–8.25)
	Q3	2.82 (0.98–8.10)
	Q4	**8.33 (2.63–26.41)**
Serving refusal	Q2	1.17 (0.39–3.55)
	Q3	1.19 (0.39–3.64)
	Q4	2.23 (0.55–9.09)
Encouragement to eat	Q2	0.51 (0.18–1.45)
	Q3	0.76 (0.28–2.07)
	Q4	0.59 (0.20–1.80)
Request-service gap	Q2	0.93 (0.36–2.34)
	Q3	1.14 (0.43–2.99)
	Q4	1.21 (0.43–3.45)
Request intensity	Q2	0.69 (0.25–1.90)
	Q3	1.89 (0.62–5.77)
	Q4	0.64 (0.23–1.80)
Snacking frequency	Q2	1.38 (0.61–3.14)
	Q3	0.52 (0.19–1.47)
	Q4	1.40 (0.58–3.39)
Meal frequency	Q2	0.78 (0.26–2.38)
	Q3	1.40 (0.55–3.58)
	Q4	0.92 (0.36–2.35)
Intrahousehold food allocation behaviors		
Meal serving order	Q2	0.42 (0.14–1.25)
	Q3	0.71 (0.28–1.82)
	Q4	0.55 (0.20–1.50)
Channeling	Q2	2.15 (0.82–5.60)
	Q3	2.47 (0.98–6.23)
	Q4	2.21 (0.90–5.42)
Shared plate	Q2	1.05 (0.43–2.61)
	Q3	0.82 (0.29–2.38)
	Q4	1.88 (0.65–5.42)
Inter-plate food sharing	Q2	0.95 (0.34–2.64)
	Q3	2.01 (0.61–6.66)
	Q4	3.23 (0.95–11.42)
Meal attendance		**0.48 (0.24–0.95)**
Other child care behaviors		
Positive social	Q2	0.47 (0.14–1.59)
	Q3	1.25 (0.46–3.37)
	Q4	0.61 (0.19–1.96)
Negative social	Q2	0.94 (0.33–2.72)
	Q3	**2.58 (1.02–6.53)**
	Q4	1.97 (0.68–5.67)
Positive health	Q2	0.99 (0.36–2.74)
	Q3	1.02 (0.40–2.56)
	Q4	**0.34 (0.12–0.94)**

[1]Risk of vitamin A deficiency based on previous clinically confirmed history of xerophthalmia.
[2]Value may be a proportion, scale value, score or count.
[3]Cut-offs selected at the quartile of each behavioral variable.
[4]Bolded odds ratios (and 95% confidence intervals) exclude unity.

xerophthalmia, with cases being half as likely as controls to eat in meals with greater attendance. Larger meals may indicate more younger children present, with whom the focus child may favorably compete for food, or the presence of adults who preferentially allocate foods to children.

This investigative approach has identified behaviors suggestive of child neglect both during feeding and with respect to other aspects of child care and caregiver–child nurturing that may directly influence the quality of a child's diet (e.g., less frequent automatic serving forcing a child to fend for itself at meals and snacks) and exposure to infection or adequacy of treatment or care once ill (e.g. more frequent scolding, less attention to hygiene and other aspects of positive care). These findings provide fresh insight into behaviors that may be underlying already documented dietary exposures associated with xerophthalmia, such as earlier weaning from the breast (Khatry *et al.*, 1995), and less frequent consumption of vitamin A rich foods (Shankar *et al.*, 1996; Sommer and West, 1996). These behavioral patterns may provide new targets for child nutrition and care education interventions that seek to strengthen family attributes that could reduce dietary and morbidity risk factors for vitamin A deficiency. Future papers will address how each behavioral variable relates to specific foods, partitioned by dietary quality. Further research needs to be conducted to more precisely determine how these behaviors are translated into health outcomes.

Many of the behavioral variables developed for this paper did not significantly influence risk of xerophthalmia. Part of the explanation for this may be due to the relatively poor reliability of the scales. In fact, those scales with the lowest reliability (serving refusals, encouragement to eat), tended to not have a significant association with past xerophthalmia status, while more reliable scales (negative social behaviors, positive health behaviors), were more likely to be associated with risk of xerophthalmia. Better operationalization of some of the scales may improve their ability to predict diet-related health outcomes.

The best way to describe intrahousehold behavior that led to xerophthalmia would have been to follow (i.e. observe) a very large cohort of children, and wait for sufficient numbers to develop xerophthalmia. As only 2–4% of children are xerophthalmic in this region of Nepal, this solution is an ideal that just was not feasible. In our study, all children in all households were treated with 200,000 IU capsules of vitamin A following the initial screening, so no children were xerophthalmic at the time of the observations. Instead, we assumed that current behavior patterns and treatment of the focus child by caregivers is similar to the period during which he or she became xerophthalmic.

One way we have tried to overcome this limitation is by collecting information on the younger sibling, as another proxy for how focus children may have been fed and treated prior to developing xerophthalmia. The validity of this approach is based on an observed lower risk of having xerophthalmia below two years of age (Katz *et al.*, 1995; Khatry *et al.*, 1995), coupled with a 13-fold (age-adjusted) increased risk of xerophthalmia among young siblings of cases compared to children with no history of xerophthalmia (Katz *et al.*, 1993).

It appears that younger siblings did differ from the focus child in patterns of behavioral variables. The younger sibling was much more likely to be automatically served food than the focus child, and was somewhat less likely to be refused. They are less likely to snack, probably because they are younger and are less able to serve themselves. Younger siblings are channeled fewer foods than the focus child, but are just as likely to eat on a shared plate as the focus child. This may mean the younger sibling is at a competitive disadvantage for food when compared with the focus child, but may also be related to age, as the youngest younger siblings were still receiving breastmilk. Younger siblings tended to receive much better treatments in terms of the summary variables developed to assess other child care behaviors. They are more likely to receive positive social behaviors and less likely to be treated negatively. They are also more likely to be receive positive health behaviors.

On the other hand, correlation patterns (Tables 2–4) were very similar between the focus child and his/her younger sibling. It appears likely that differences in treatment of the focus child and the younger sibling are primarily due to differences in age. These findings reinforce the usefulness of both sets of information, current focus child treatment and current younger sibling treatment, for assessing behavioral patterns within the household that produced a xerophthalmic child in the past.

The study has some limitations. The first lies in the use of the reactivity score. We adjusted for reactivity effects by using the mean reactivity score for all household observations; a more powerful adjustment would be to adjust each observation individually. On the other hand, to the best of our knowledge, this is the first time that a study of human health behavior has attempted to adjust for reactivity effect. Increasingly, the structured observation method itself has been studied in terms of its appropriateness as a tool for estimating health behavior, and several recent studies have examined the reactivity issue (Cousens *et al.*, 1996; Gittelsohn *et al.*, 1997). Within a household, observed behaviors show considerable variability from observation period to observation period. Some of this variability may be explained by differences in time of day and season. Other aspects of behavioral

variability from a true behavioral norm appear related to reactivity, especially during the initial period of observation (day, hour, visit), when reaction to the presence of the observer may be greatest. After that initial period of acclimation to the presence of the observer, reactive behaviors, such as cleaning the home for the observer, tend to fall off. Future efforts should continue to measure the effect of reactivity on structured observations of behavior, and how to compensate for that effect.

Finally, no information was collected on child appetite independent of amount of food consumed – although several of our variables (request intensity, second helpings) could be used to construct a variable similar to the child demand scale developed by Engle and Zeitlin (1996).

In conclusion, we found that examining intrahousehold behavior is critical for understanding the causes of vitamin A deficiency in rural Nepalese children. The direct observation of household behavior has great potential for identifying possible new interventions to improve children's diets and care in the future.

Acknowledgements—The study was funded through Cooperative Agreement No. DAN 0045-A-00-5094-00 between the Center for Human Nutrition of the Department of International Health/Dana Center for Preventive Ophthalmology, The Johns Hopkins University and The Office of Health and Nutrition, U.S. Agency for International Development, Washington, DC, with additional support from Task Force Sight and Life (Roche, Basel), and NIH shared instrument grant No. S10-RR 04060. We would like to acknowledge the key assistance of Chandra Dhungel and Birendra Dahal in the design and implementation of this component of the research. We would also like to acknowledge the contributions of the following project investigators and staff: Dr. Rajendra Pradhan, Stephen C. LeClerq, Dr. Subarna Khatry, Sharada R. Shrestha, D. N. Mandal, T. R. Shakya, Dr. R. P. Pokhrel, Dr. Joanne Katz, Molly Gingerich and Dr. David Caulder.

REFERENCES

Ashby, G. (1985) *Some Things of Value ... Micronesian Customs and Beliefs*, Revised Edition. Rainy Day Press, Eugene, OR.

Bentley, M. E., Stallings, R. Y., Fukamoto, M. and Elder, J. A. (1991) Maternal feeding behavior and child acceptance of food during diarrhea, convalescence, and health in the Central Sierra of Peru. *American Journal of Public Health* **81**, 43–47.

Bentley, M. E., Boot, M. T., Gittelsohn, J., and Stallings, R. Y. (1994) The use of structured observations in the study of health behaviour. Occasional Paper No. 27. IRC International Water and Sanitation Center, The Hague.

Bentley, M. E., Black, M. M. and Hurtado, E. (1995) Child-feeding and appetite: what can programmes do? *Food and Nutrition Bulletin* **16**, 340–348.

Cousens, S., Kanki, B., Toure, S., Diallo, I. and Curtis, V. (1996) Reactivity and repeatability of hygiene behavior: structured observations from Burkina Faso. *Social Science and Medicine* **43**, 1299–1308.

Cronbach, L. J. (1951) Coefficient alpha and the internal structure of tests. *Psychometrika* **16**, 297–334.

Curtis, V., Cousens, S., Mertens, T., Traore, E., Kanki, B. and Diallo, I. (1993) Structured observations of hygiene behaviors in Burkina Faso: validity, variability, and utility. *Bulletin of the World Health Organization* **71**, 23–32.

Dettwyler, K. A. (1986) Infant feeding in Mali, West Africa: variations in belief and practice. *Social Science and Medicine* **23**, 651–664.

Dettwyler, K. A. (1989) Styles of infant feeding: Parental/caretaker control of food consumption in young children. *American Anthropologist* **91**, 696–703.

DeVellis, R. F. (1991) Scale development: theory and applications. In *Applied Social Research Methods Series*, Vol. 26. Sage Publications, Inc., Newbury Park, CA.

Engle, P. L. and Zeitlin, M. (1996) Active feeding behavior compensates for low interest in food among young Nicaraguan children. *Journal of Nutrition* **126**, 808–1816.

Gittelsohn, J. (1989) *Intrahousehold Food Distribution in Rural Nepal*. University of Connecticut, University Microfilms, Ann Arbor.

Gittelsohn, J. (1991) Opening the box: intrahousehold food allocation in rural Nepal. *Social Science and Medicine* **33**, 1141–1154.

Gittelsohn, J., Shankar, A. V., West, K. P. and Pokhrel, R. P. (1994) Accuracy of direct observation for food intake estimation in rural Nepal. *Journal of the American Dietetic Association* **94**, 1273–1277.

Gittelsohn, J., Shankar, A. V., West, K. P. Jr., Ram, R., Dhungel, C. and Dahal, B. (1997) Infant feeding practices reflect antecedent risk of xerophthalmia in Nepali children. *European Journal of Clinical Nutrition* **51**, 484–490.

Katz, J., Zeger, S. L., West, K. P. Jr., Tielsch, J. M. and Sommer, A. (1993) Clustering or xerophthalmia within households and villages. *International Journal of Epidemiology* **22**, 1709–1715.

Katz, J., West, K. P., Khatry, S. K., Thapa, M. D., LeClerq, S. C., Pradhan, E. K., Pokhrel, R. P. and Sommer, A. (1995) Impact of vitamin A supplementation on prevalence and incidence of xerophthalmia in Nepal. *Investigative Ophthalmology and Visual Science* **36**, 2577–2583.

Khatry, S. K., West, K. P., Katz, J., DeClerq, S. C., Pradhan, E. K., Wu, L. S.-F., Thapa, M. D. and Pokhrel, R. P. (1995) Epidemiology of xerophthalmia in Nepal: a pattern of household poverty, childhood illness, and mortality. *Archives of Ophthalmology* **113**, 425–429.

Meldrum, B. (1984) Traditional child-rearing practices of the Oje market women of Ibadan. In *Nigerian Children: Developmental Perspectives*, ed. H. Curran, pp. 174–196. Routledge and Kegan Paul, London.

Shankar, A. V., West, K. P., Gittelsohn, J., Katz, J. and Pradham, R. (1996) Chronic low intakes of vitamin A-rich foods in households with xerophthalmic children: a case-control study in Nepal. *American Journal of Clinical Nutrition* **64**, 242–248.

Stanton, B. F. and Clemens, J. D. (1987) An educational intervention for altering water-sanitation behaviors to reduce childhood diarrhea in urban Bangladesh. II. A randomized trial to assess the impact of the intervention on hygienic behaviors and rates of diarrhea. *American Journal of Epidemiology* **125**, 292–301.

Sommer, A. (1982) *Nutritional Blindness, Xerophthalmia and Keratomalacia*. Oxford University Press, New York.

Sommer, A. and West, K. P. (1996) *Vitamin A Deficiency: Health Survival and Vision*. Oxford University Press, New York.

51

Neonatal vitamin A supplementation: effect on development and growth at 3 y of age[1-3]

Jean H Humphrey, Tina Agoestina, Ami Juliana, Seni Septiana, Hanna Widjaja, Mary C Cerreto, Lee SF Wu, Rebecca N Ichord, Joanne Katz, and Keith P West Jr

ABSTRACT We reported recently that neonatal supplementation with 52 μmol vitamin A reduced infant mortality by 64%; acute side effects were limited to a 3% excess rate of a bulging fontanelle. The current study was conducted to identify developmental changes at 3 y of age associated with neonatal vitamin A supplementation or a bulging fontanelle. Children who had a bulging fontanelle ($n = 91$) and 432 children who had normal fontanelles after receiving vitamin A or placebo were evaluated with the Bayley Scales of Infant Development. Mean scores for the mental, psychomotor, and behavioral rating scale (BRS) plus 3 subscales of the BRS were not significantly different for treatment-fontanelle-specific groups. In regression models predicting each score, a bulging fontanelle had a small negative effect in all models; when 1 child who was injured from birth was removed from the analysis the effect of a bulging fontanelle was not significant in any model ($P \geq 0.35$). Vitamin A supplementation had a small beneficial effect on all developmental scores, which was significant for one of the BRS subscales (orientation-engagement) and also for a second (motor quality) when the outlier child was removed. Compared with children with normal fontanelles in the placebo group, children with a bulging fontanelle in the vitamin A group tended to grow less (−0.5 cm, $P = 0.33$), whereas those with normal fontanelles in the vitamin A group grew significantly more (0.68 cm, $P < 0.05$), over the first 3 y of life. This study provides no evidence that neonatal vitamin A supplementation is associated with biologically significant adverse growth or developmental sequelae. *Am J Clin Nutr* 1998;68:109–17.

KEY WORDS Vitamin A, neonates, bulging fontanelle, Bayley Scales of Infant Development, supplementation, growth

INTRODUCTION

Vitamin A deficiency is a major public health problem throughout the developing world, affecting an estimated 124 million young children and accounting for ≥1 million child deaths each year (1). Periodic high-dose vitamin A supplementation of preschool children >6 mo of age, an integral part of health programs in many countries, is well tolerated (2) and results in 20–50% reductions in mortality (3).

Supplementation of neonates has been suggested as a feasible approach to bolstering body stores of vitamin A in early infancy (4, 5). Initial studies of the effect of supplementation of young infants on mortality were inconclusive (3) or reported no significant reduction (6). More recently, however, we reported results from a trial including 2067 Indonesian neonates in which a single 52-μmol oral dose of vitamin A administered on the first day of life reduced infant mortality by 64% (7).

Tolerance of large oral doses of vitamin A by young infants has been assessed as a part of our trial (8) and by several other investigators (9–13; O Thanangkul, C Promkutkaew, T Waniyapong, D Damrongsak, unpublished observations, 1974). Bulging of the anterior fontanelle is clearly the most common acute side effect in infants given vitamin A: 5 of 7 studies reported excess rates of this sign (8, 9–11, 13). These reports describe vitamin A–induced bulging fontanelles as being isolated, ie, not associated with vomiting, irritability, or other morbidity (8, 13); transient (9, 11, 13); and not associated with increased intracranial pressure (8), leading some to conclude that they are benign (14).

Lingering concerns remain about the long-term safety of neonatal vitamin A supplementation, especially when accompanied by a bulging fontanelle (15). Given the promise that neonatal vitamin A supplementation may hold for infant survival, we conducted the present study to search for developmental changes at 3 y of age that might be associated with neonatal vitamin A supplementation or bulging fontanelles.

SUBJECTS AND METHODS

Study population

The study population was selected from among infants who had participated in the newborn vitamin A supplementation trial described previously (7, 8). Briefly, all infants born at Hasan

[1] From the Center for Human Nutrition, The Johns Hopkins School of Hygiene and Public Health, Baltimore; Hasan Sadikin Hospital and Padjadaran University (Faculties of Medicine and Psychology), Bandung, Indonesia; and the Department of Neurology, The Johns Hopkins School of Medicine, Baltimore.

[2] Supported by Cooperative Agreement no. DAN-0045-A-5094 between Johns Hopkins University and the Bureau of Research and Development, Office of Health and Nutrition, US Agency for International Development, Washington, DC, and NIH grant S10-RR04060.

[3] Address reprint requests to J Humphrey, The Johns Hopkins School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205. E-mail: humphrey@zvitambo.icon.co.zw.

Received August 22, 1997.
Accepted for publication January 11, 1998.

The American Society for Clinical Nutrition Inc. does not endorse any commercial enterprise.

52

Sadikin Hospital in Bandung, Indonesia, between 18 June 1992 and 2 June 1993 were considered for enrollment in the trial. Infants with very low birth weights (< 1500 g) or other life-threatening conditions were excluded. The study protocol was approved by the National Institute of Health, Research, and Development of the Indonesian Ministry of Health of Indonesia, the Ethical Review Committee of Hasan Sadikin Hospital, and the Joint Committee on Clinical Investigation of The Johns Hopkins School of Medicine.

A total of 2067 infants were enrolled after their parents provided written, informed consent. The baseline examination included taking a history of morbidity since birth and palpating the anterior fontanelle. The first 973 infants also underwent a duplex Doppler examination of the anterior cerebral artery by cranial ultrasound so that we could measure the resistive index, an indirect indicator of intracranial pressure (16). After the baseline examination, 1034 infants received 1 oral dose of 52 μmol vitamin A with 23 μmol vitamin E and 1033 infants received placebo (vitamin E only) by random allocation. Infants were monitored at frequent intervals by nurses and were reexamined by a pediatrician 24 and 48 h after dosing. Bulging of the fontanelle was the only side effect of vitamin A supplementation: 77 (7.4%) of the vitamin A–treated infants compared with 45 (4.4%) of the placebo-treated infants had a bulging fontanelle 24 or 48 h or both postdosing. Bulging of the fontanelle was not associated with any other sign or symptom (eg, fever, irritability, vomiting, or diarrhea) nor with an increase in intracranial pressure, as reflected by the resistive index. Thus, acute side effects of a 52-μmol dose of vitamin A were limited to an isolated bulging fontanelle in a small proportion of neonates.

At 1 y of age, infants were visited in their homes by study nurses. Vital status could be confirmed for 1839 (89%) of the infants. Infant mortality was reduced by 64% among vitamin A recipients compared with placebo-treated infants: there were 7.2 compared with 19.8 deaths per 1000 child-years in the control and vitamin A groups, respectively (7). Nearly all the effect was confined to deaths occurring between 1 and 4 mo of age. Health care visits for cough and fever [a symptom and sign of pneumonia, the leading cause of death among young infants in developing countries (17–19)] were reduced by 73% and 51%, respectively, over the same first 4 mo of life in a subgroup followed at closer intervals (7).

Follow-up of development and growth

The present study was conducted to evaluate mental and motor development and growth at 3 y of age among the following groups of children: those who had a bulging fontanelle (BF) either 24 or 48 h after receiving either 52 μmol vitamin A (VA) or placebo (PL) and those whose fontanelle was normal (NF) after dosing. Children were selected in the following way.

Children who had a bulging fontanelle after dosing

Of the 2067 infants enrolled, 122 (45 PL and 77 VA) developed a bulging fontanelle after dosing. Of these 122, 14 (11.5%, 4 PL and 10 VA) were lost to follow-up and 1 (0.82%, VA) died by 1 y of age. The remaining 107 children (41 PL-BF and 66 VA-BF) were selected for this study.

Children who had a normal fontanelle after dosing

For each BF child, 4 NF children were selected, 2 from each treatment group, matched to the BF children by sex, age (within 2 wk), birth weight (within 200 g), and years of paternal education. These clusters of 5 children (1 BF + 4 NF) were therefore selected to be similar for major factors frequently associated with development to enhance our power to detect differences in development due to either vitamin A supplementation or a bulging fontanelle.

Instrument

The Bayley Scales of Infant Development, second edition (BSID II) (20), were used to evaluate development. The BSID II has 3 individually scored sections: the mental scale, motor scale, and behavioral rating scale (BRS). The mental scale assesses memory, habituation, problem solving, early number concepts, generalization, classification, vocalizations, language, and social skills and yields the mental development index (MDI). The motor scale assesses control of gross and fine muscle groups and includes tasks such as running, jumping, use of a pencil, and imitation of hand movements and yields the psychomotor development index (PDI). Both the MDI and PDI can be expressed as raw scores or as age-adjusted index scores. Because all of the children in our study were the same age at the time of testing, our results are presented as raw scores, except where noted.

The BRS is new to the BSID II. It assesses qualitative aspects of the child's behavior while he or she is taking the mental and motor tests. The total BRS is a summary of 3 independent subscales:

1) Orientation-engagement: an assessment of the child's proclivities toward approaching or avoiding environmental interactions that are task related or social; low scores are meant to identify apathetic, passive, low-energy, fearful children who fail to socially engage with the tester.

2) Emotional regulation: an assessment of the child's activity, adaptability, affect, cooperation, persistence, and frustration tolerance; low scores are meant to identify uncooperative, irritable, easily frustrated children.

3) Motor quality: an assessment of muscle tone, fine and gross motor control, and quality of movement; low scores suggest probable neuromotor dysfunction.

A key goal in the revision of the first edition of the BSID was to extend the maximum testable age from 30 to 42 mo. This allowed us to use the instrument in assessing 3-y-old children. In Indonesia, the first edition of the BSID has been used extensively (21–23). However, to our knowledge, this was the first time the BSID II was used in testing Indonesian 3-y-olds. Therefore, the test items for this age group required translation and minor cultural adaptation. This was done with written permission of the Psychological Testing Corporation (San Antonio, TX).

Training and reliability testing

Two Indonesian psychologists with master's degrees (AJ and SA), both of whom were trilingual (Indonesian, Sudanese, and English) and experienced with children, administered all tests. They underwent intensive training by a senior Indonesian psychologist (HW) and an American psychologist with extensive experience in the BSID II (MCC). Although language and minor cultural modifications were made as mentioned previously, the standard techniques described in the BSID II manual were followed rigorously when administering all items. To further minimize within-tester and between-tester variability, we increased the standardization of some test items. For example, for item 173 (building a "T" with 5 blocks) the 2 psychologists were trained to place the blocks in exactly the same order (vertical line first, then tails).

To monitor reliability under study conditions, 50 study children who were scheduled during the first few months of the study were asked to return 1 wk after their scheduled test to be retested. Both tests were conducted within 2 wk of the child's third birthday in a special clinic dedicated to the study located in Hasan Sadikin Hospital. Thirty of these children were tested twice by the same psychologists (15 by each psychologist) to provide an estimate of within-tester agreement for each psychologist, and 20 were tested once by each psychologist (10 were tested first by AJ, 10 were tested first by SS) to provide an estimate of between-tester agreement.

Protocol

All children were tested within 2 wk of their third birthday. About 1 mo before each child's birthday, a letter was mailed to the family explaining the study, giving the date of the proposed developmental test, and requesting their participation. A consent form was included to provide ample time for review. On the designated day, 2 study nurses traveled to the child's home and obtained written, informed parental consent. The nurses elicited a 1-wk morbidity history and conducted a simple physical examination to identify children with acute or recent illness (eg, cough, upper respiratory tract infection, or fever) that might interfere with developmental testing. Healthy children were transported to the study clinic with a parent. For ill or convalescing children, testing was delayed until recovery, but was always within 2 wk of the child's third birthday.

To control for any differences between psychologists, all 5 children in a cluster (1 BF and 4 matched NF) were assigned to the same psychologist for testing. Cluster assignments were balanced across psychologists by treatment group and birth month of the BF child. Children were tested in their homes if they lived too far to be conveniently transported to and from the clinic or if they were too uncomfortable in the clinic to be tested. Some children required > 1 session to complete the test. Additional sessions were scheduled as soon after the initial session as possible, as recommended in the BSID II manual, and were always within 2 wk of the child's birthday. The place of examination and the number of sessions were recorded.

The psychologists, nurses, and all other investigators and study personnel in Indonesia remained masked to the treatment group, fontanelle status, and cluster of all children until the study was completed. If a selected NF child could not be located, refused to participate, or was too ill to be tested within 2 wk of his or her third birthday, a replacement child was selected by one of us in Baltimore. If a BF child was lost to follow-up, an NF child was selected as the replacement, matched with another BF child to preserve the masking of the Indonesian investigators to the fontanelle status of the children. In this way, some clusters had a fifth NF child included in the analysis.

After developmental testing, study nurses measured weight with an upright balance beam scale and height with a stadiometer (Shorr Inc, Olney, MD). Anthropometric measurement techniques were standardized at the beginning of the study and midway through the study.

Statistical methods

Repeated tests of the same child were compared by using Pearson's correlation coefficient (r) and intraclass correlation (ρ^2) to estimate within-tester and between-tester agreement. Baseline characteristics that were potential confounders of the effect of vitamin A supplementation or a bulging fontanelle on growth and development were compared across the 4 treatment-fontanelle-specific groups by analysis of variance (ANOVA) for means and chi-square for proportions. In the same way, baseline characteristics of children with a bulging fontanelle who were lost to follow-up were compared with those of children included in this study. Mean developmental scores for treatment-fontanelle-specific groups were compared by using ANOVA.

General linear regression modeling was used to estimate the effects of treatment and fontanelle status on developmental scores after adjusting for potentially confounding variables. Covariates for these models were at least of borderline significance ($P < 0.08$) in univariate and trivariate (including treatment and fontanelle status) models in which the outcomes were the developmental scores. In addition, 2 groups of covariates were identified that were strongly associated with developmental scores in univariate and trivariate analyses and were also strongly correlated with each other. Paternal and maternal education are both indicators of the family's socioeconomic status and, as expected, these 2 variables were closely correlated in our study ($r^2 = 0.70$). Similarly, birth weight, birth length, and gestational age are all indicators of intrauterine growth and were correlated with each other in our study (r^2 = 0.71, 0.34, and 0.27 for correlations between weight and length, weight and gestational age, and length and gestational age, respectively). To determine whether maternal or paternal education had the stronger independent effect on development, both were included in the same model. When this was done, the β coefficient and significance level associated with maternal education were greater than those for paternal education for all models. Thus, only maternal education was retained in the final models. Similarly, birth weight, birth length, and gestational age were included together in each model and the one with the smallest associated P value was retained in each of the final models. If, in predicting a given developmental score, none of the indicators of intrauterine growth were significant, none were included in the final model.

Test score distributions were examined for each treatment-fontanelle-specific group and 11 children were identified as having outlying values on ≥ 1 test. The 6 final general linear regression models were rerun 11 times, each time excluding 1 of these children. Excluding 10 of these children from the analyses did not change the magnitude or significance level of either the vitamin A treatment or bulging fontanelle effect in any model. However, exclusion of 1 VA-BF child substantially enhanced the beneficial effect of vitamin A and reduced the adverse effect of a bulging fontanelle in 3 models: MDI, PDI, and motor quality. This child was a second-born twin, delivered at the hospital by cesarean section because of a transverse lie with a prolapsed fetal arm 3 h after her brother had been delivered vaginally in a rural village. Apgar scores for this child at 1 and 5 min were 4 and 8, respectively. By maternal history, the child had right hemiparesis (decreased arm and leg mobility) from birth and subsequently had 3 febrile illnesses accompanied by seizures at 6, 18, and 34 mo of age. This history is most consistent with a prenatal brain injury (24), although infectious illnesses with central nervous system involvement may have also contributed to her developmental delay. However, perinatal insult (before or after vitamin A dosing) cannot be ruled out from the available evidence, so we present the data with and without data from this outlier. All tests were two tailed. P values ≤0.05 were considered significant.

54

RESULTS

Between-tester and within-tester reliability

The between-tester agreement data for the repeated testing of 20 children by both psychologists are shown in **Table 1**. The within-tester agreement data for each psychologist for the repeated testing of 15 children are shown in **Table 2**. For comparison, interscorer agreement and within-tester values obtained by examiners during standardization of the BSID II (20) are also presented. The correlation coefficient for between-tester agreement attained by our psychologists was somewhat lower than that for interscorer agreement reported in the BSID II manual, probably because of 2 reasons. First, interscorer agreement (used in the standardization of the BSID II) is measured by having the first tester administer the scales and score and a second tester observe the testing and also score. Hence, interscorer agreement measures the ability of 2 testers to assign the same score to the same observed behavior. Between-tester agreement (used in our study) measures the agreement between scores assigned by 2 testers during separate administrations of the test. Thus, our between-tester reliability exercise assessed not only intertester agreement, but also test-retest reliability, so its correlation coefficients are likely to be lower. Second, by chance, the children tested for the between-tester reliability study had very similar, tightly distributed test scores, weakening the linear correlation. The very high intraclass correlations are a better reflection of between-tester reliability in this sample.

Within-tester agreement was excellent for psychologist B and acceptable, although lower, for psychologist A (Table 2). MDI and PDI scores increased by an average of 5.6 and 2.8 points, respectively, between the first and second test. These changes are greater than the 2-point average increases observed in the standardization population reported in the BSID II manual, despite our use of a longer retest interval (7 compared with 4 d). This difference may have been due to a greater practice effect in our older population.

Retention and conditions of testing

Of the 107 (66 VA, 41 PL) BF children, 91 (85%) [58/66 (88%) VA and 33/41 (80%) PL] were located at 3 y of age and underwent developmental testing. Of the 428 NF children originally selected (214 VA, 214 PL), 378 (88%) were tested. For an additional 41 (10%) NF children, appropriate replacements were recruited. Thirteen additional NF children were recruited to replace BF children lost to follow-up. Thus, a total of 432 NF children (215 VA, 217 PL) were included in the study. Among the 1034 vitamin A–treated children in the original trial, mean birth weight was 89 g greater for those who developed a bulging fontanelle than for those who did not ($n = 957$, $\bar{x} \pm SD = 2995.5 \pm 441.8$ g, compared with $n = 77$, $\bar{x} \pm SD = 3084.7 \pm 357.6$ g, respectively). Given the large sample size, this difference was significant ($P = 0.04$). However, because birth weight was one of our matching variables for this developmental follow-up study, there were no significant differences in birth weight among the 4 treatment-fontanelle-specific groups, nor were there differences in any other baseline characteristic examined (**Table 3**).

Of the 67 children lost to follow-up, 54 could not be located (27 per treatment group), 5 refused to participate, 4 died between 1 and 3 y of age (1 PL and 3 VA, all NF), 2 were too ill to test within 2 wk of their third birthday (1 in each treatment group), and 2 were "untestable" (unable to complete the test; both PL-NF). A third child (PL-NF) was untestable for the psychomotor scale only.

Of the 122 children with a bulging fontanelle identified in the original trial, there were no significant differences between the 91 children examined in this developmental assessment and the 31 lost to follow-up over the 3-y period in birth weight, birth length, mean 5-min Apgar score, maternal parity, proportion male, and proportion appropriate for gestational age ($P \geq 0.34$), although lost cases tended to have younger mothers ($P = 0.11$).

Of all the children, 80% were tested in the hospital clinic; the remainder were tested in their homes. There were no differences in the proportion of children tested at home by treatment group ($P > 0.23$), fontanelle status ($P > 0.94$), or psychologist ($P > 0.70$). Most children [444 (85%)] could be tested in a single session. There were no differences in the proportions of children tested in 1 session compared with > 1 session by treatment group ($P > 0.63$), fontanelle status ($P > 0.83$), or psychologist ($P > 0.76$).

There were no differences according to treatment group or fontanelle status in the proportions of children with a positive 1-wk history of diarrhea, fever, cough, ear infection, or fast or difficult breathing. Furthermore, neither a history of any illness nor identification of a current sign or symptom by the study nurse was associated with any developmental score. This suggests that the nurses did a good job in identifying children who had illnesses severe enough to interfere with testing and in delaying these children's developmental examinations.

TABLE 1
Between-tester agreement for repeated testing of 20 children[1]

Scale	BSID II reference population (interscorer agreement): r	Psychologist A	Psychologist B	r	ρ^2
MDI	0.96	107.1 ± 6.9[2]	106.0 ± 6.7	0.53	0.96
PDI	0.75	107.2 ± 6.2	105.8 ± 6.2	0.73	0.98
Orientation-engagement	0.82	37.8 ± 3.7	38.0 ± 3.7	0.53	0.99
Emotional regulation	0.83	41.8 ± 3.8	40.6 ± 3.3	0.74	0.75
Motor quality	0.79	35.0 ± 1.1	35.3 ± 1.4	0.75	0.85
Total BRS	0.88	112.3 ± 7.0	111.5 ± 6.4	0.71	0.85

[1] BSID II, Bayley Scales of Infant Development, second edition (20); MDI, mental development index; PDI, psychomotor development index; BRS, behavioral rating scale. r is Pearson's correlation coefficient and ρ^2 is the intraclass correlation, $\sigma^2/(\sigma_x^2 + \sigma_e^2)$, where σ_x is between-children variance and σ_e is within-child variance.

[2] $\bar{x} \pm SD$.

TABLE 2
Within-tester agreement for repeated testing of 15 children by each psychologist[1]

Scale	BSID II reference population		Psychologist A				Psychologist B			
	r	ρ^2	Time 1	Time 2	r	ρ^2	Time 1	Time 2	r	ρ^2
MDI	0.91	0.89	103.2 ± 8.9[2]	109.6 ± 7.9	0.76	0.79	103.9 ± 8.5	108.7 ± 7.8	0.92	0.84
PDI	0.79	0.81	107.6 ± 6.5	110.5 ± 5.8	0.71	0.78	103.9 ± 11.3	106.7 ± 9.8	0.95	0.76
Orientation-engagement	0.61	0.87	34.7 ± 5.8	36.3 ± 5.3	0.86	0.82	36.7 ± 5.6	38.6 ± 5.0	0.83	0.79
Emotional regulation	0.66	0.86	40.1 ± 6.6	42.7 ± 4.0	0.79	0.38	40.1 ± 5.5	41.5 ± 4.1	0.84	0.56
Motor quality	0.71	0.80	35.0 ± 1.4	35.8 ± 1.2	0.65	0.78	35.3 ± 1.9	35.7 ± 1.8	0.92	0.92
Total BRS	0.60	0.89	108.0 ± 11.2	112.6 ± 8.0	0.83	0.51	110.0 ± 11.1	113.5 ± 9.2	0.89	0.68

[1] BSID II, Bayley Scales of Infant Development, second edition (20); MDI, mental development index; PDI, psychomotor development index; BRS, behavioral rating scale. r is Pearson's correlation coefficient and ρ^2 is the intraclass correlation, $\sigma^2/(\sigma_x^2 + \sigma_e^2)$, where σ_x is between-children variance and σ_e is within-child variance.
[2] $\bar{x}$ ± SD.

Association of developmental scores with vitamin A treatment and fontanelle status

Mean developmental scores, stratified by treatment group and fontanelle status during the first 48 h of life, are presented in **Table 4** and **Table 5**. Because testing conditions may affect test scores, data are presented separately for children tested in the study clinic (Table 4) and children tested at home (Table 5). The VA-BF child who was considered to be an outlier (*see* the "Statistical methods" section) was tested at home; Table 5 is presented with and without the data from this child. Children tested at home had significantly lower scores than did children tested in the clinic ($P \leq 0.02$). However, scores by treatment group and fontanelle status were not significantly different for any developmental score among those tested at home or in the clinic. In comparing the 44 VA-BF children with the 175 VA-NF children tested in the clinic, we had 87% power to detect a 1.5-point difference in raw scores on the MDI and 99% power to detect a 1.0-point difference in raw scores on the PDI between the BF and NF children. These differences are approximately equal to a 3-point difference in age-adjusted index score, or 0.2 of the reference population SD. We had ≥97% power to detect a difference of 1.5 points in each of the BRS factor scores and 89% power to detect a 2.5-point difference in the total BRS score.

The proportions of children classified as significantly delayed, mildly delayed, within normal limits, and accelerated (MDI and PDI age-adjusted index scores ≤69, 70–84, 85–114, and ≥115, respectively) were not significantly different across all treatment-fontanelle-specific groups (**Table 6**). In addition, these distributions were similar to those of the US reference population used to standardize the BSID II. Four children (0.7%) were significantly delayed according to either the mental scale, psychomotor scale, or both, 1 from each treatment-fontanelle-specific group.

To further explore the data, we calculated the proportion of children failing each item of the mental and motor scales for the 4 treatment-fontanelle-specific groups. Significant differences were found only for 2 items on the motor scale. For item 96, "copies circle," 0.5%, 1.8%, and 0.5% of children in the VA-NF, VA-BF, and PL-NF groups failed compared with 9.1% in the PL-BF group ($P < 0.003$). For item 97, "uses eye-hand coordination in tossing ring," none of the children in the VA-NF and VA-BF groups and 1.4% of children in the PL-NF group failed compared with 6.1% in the PL-BF group ($P < 0.02$). Item analysis for the BRS was carried out by comparing the proportion of children scoring 5 (the highest score) for each item across the 4 groups. Significant differences were found between

TABLE 3
Baseline characteristics of the sample assessed for development at 3 y of age by treatment group and fontanelle status[1]

Characteristic	Vitamin A		Placebo	
	Bulging fontanelle (n = 58)	Normal fontanelle (n = 215)	Bulging fontanelle (n = 33)	Normal fontanelle (n = 217)
Male (%)	55.17	50.70	54.55	52.53
Birth weight (g)	3067.3 ± 364.1[2]	3063.0 ± 372.4	3005.2 ± 363.4	3042.1 ± 349.8
Birth length (cm)	49.0 ± 1.2	48.6 ± 1.8	48.2 ± 1.8	48.5 ± 1.4
Gestational age (%)				
<38 wk	3.45	3.72	6.06	4.61
38–42 wk	96.55	96.28	93.94	95.39
>42 wk	0	0	0	0
Appropriateness of birth weight for gestational age (%)				
Appropriate	94.83	95.35	100.00	97.24
Small	1.72	1.86	0	1.84
Large	3.45	2.79	0	0.92
5-min Apgar score	9.0 ± 0.4	9.0 ± 0.4	9.1 ± 0.3	8.9 ± 0.8
Maternal age (y)	27.7 ± 6.3	27.9 ± 6.0	28.2 ± 5.8	27.7 ± 5.3
Maternal parity	2.6 ± 2.2	2.6 ± 2.1	2.5 ± 1.7	2.4 ± 1.7
Maternal education (y)	8.60 ± 3.26	9.36 ± 3.67	9.42 ± 3.29	9.51 ± 3.38

[1] None of the characteristics were significantly different among groups, $P > 0.10$.
[2] $\bar{x}$ ± SD.

TABLE 4
Developmental scores on the BSID II by treatment group and fontanelle status for children tested in the study clinic[1]

	Raw MDI	Raw PDI	Total BRS	Orientation	Emotional regulation	Motor quality
Vitamin A						
BF (n = 44)	157.43 ± 4.80	102.41 ± 2.36	115.16 ± 7.59	39.73 ± 3.52	42.11 ± 4.26	35.73 ± 1.74
NF (n = 175)	158.15 ± 4.21	102.39 ± 1.78	115.19 ± 6.94	39.40 ± 3.73	42.56 ± 4.02	35.70 ± 1.33
Total vitamin A (n = 219)	158.00 ± 4.33	102.39 ± 1.91	115.18 ± 7.06	39.46 ± 3.68	42.47 ± 4.06	35.70 ± 1.42
Placebo						
BF (n = 27)	157.07 ± 4.58	101.41 ± 3.25	112.96 ± 9.13	37.92 ± 4.62	42.18 ± 4.95	35.18 ± 1.75
NF (n = 173)	157.59 ± 6.20	102.14 ± 2.85	114.54 ± 7.66	39.00 ± 3.60	42.55 ± 4.34	35.44 ± 1.79
Total placebo (n = 200)	157.52 ± 6.00	102.04 ± 2.91	114.32 ± 7.87	38.86 ± 3.76	42.50 ± 4.41	35.41 ± 1.78
Both treatment groups						
BF (n = 71)	157.30 ± (4.68)	102.03 ± 2.76	114.32 ± 8.21	39.04 ± 4.04	42.14 ± 4.50	35.52 ± 1.71
NF (n = 348)	157.87 ± (5.29)	102.27 ± 2.37	114.86 ± 7.30	39.20 ± 3.66	42.55 ± 4.17	35.57 ± 1.58

[1] $\bar{x}$ ± SD. BSID II, Bayley Scales of Infant Development, second edition (20); MDI, mental development index; PDI, psychomotor development index; BRS, behavioral rating scale; BF, bulging fontanelle; NF, normal fontanelle.

groups for item 28, "slow and delayed movement." The proportion of children scoring 5 was 95.8%, 93.0%, 88.9%, and 91.9% for the VA-NF, VA-BF, PL-NF, and PL-BF groups, respectively ($P < 0.05$). When the analyses were repeated without stratifying for fontanelle status, significantly more vitamin A–treated children scored 5 than did placebo-treated children (95.2% compared with 89.2%, $P < 0.02$).

General linear regression models for each developmental score are presented in **Table 7**. Models including only fontanelle status and treatment group are presented first (model A). Model B adjusts for other important covariates and model C presents the model B analysis after excluding the outlier. Maternal education was a strong predictor of all 6 developmental scores, and gestational age was an important predictor in 4 of the models. The estimated effect of having a bulging fontanelle (after either placebo or vitamin A treatment) was negative in the models of all developmental scores (suggesting a detrimental effect) but was small in magnitude (<0.7 points) and was not significant in any model except for PDI, which was strongly influenced by the outlier. When the outlier was excluded (raw psychomotor development index C), the effect of the bulging fontanelle on the PDI was −0.26 points and was not significant ($P = 0.35$).

The parameter estimate for treatment was positive in all 6 models (suggesting a beneficial effect of vitamin A treatment). Although this effect was not large in any model, the difference was significant in the model for orientation-engagement ($P = 0.04$) and reached borderline significance ($P = 0.10$) for motor quality and the total BRS. When the outlier was excluded from the analyses, the beneficial effect of vitamin A reached or approached significance for motor quality ($P = 0.05$) and for the PDI ($P = 0.07$) and remained significant for orientation-engagement ($P = 0.04$). The interaction term between treatment and fontanelle status was not significant in any model, indicating that the effect of vitamin A on developmental scores was not different for BF compared with NF children.

Association of vitamin A and fontanelle status with growth

Wasting was rare in this study population: the mean weight-for-height z score was −0.23 and 9.1% of the children were below the 10th National Center for Health Statistics percentile for weight-for-height (25). Rates and degrees of wasting did not differ across treatment-fontanelle-specific groups ($P > 0.76$). Mean change in ponderal index [weight (g) × 100/length^3 (cm)] (26) between birth and 3 y also did not differ across treatment-fontanelle-specific groups ($P > 0.16$).

Conversely, as a group, these children experienced linear growth retardation over the first 3 y of life. Their z scores fell from −0.72 for length-for-age at birth to −1.63 for height-for-age at 3 y. The interaction term for treatment and fontanelle status was significant in a model predicting change in stature,

TABLE 5
Developmental scores on the BSID II by treatment group and fontanelle status for children tested in their homes[1]

	Raw MDI	Raw PDI	Total BRS	Orientation	Emotional regulation	Motor quality
Vitamin A						
BF (n = 14)	157.00 ± 8.07	98.93 ± 10.72	108.86 ± 8.67	36.43 ± 4.77	40.57 ± 4.59	34.07 ± 3.56
BF (outlier excluded)	158.69 ± 5.20	101.77 ± 1.48	109.54 ± 8.63	36.31 ± 4.94	40.15 ± 4.49	34.92 ± 1.66
NF (n = 40)	155.62 ± 4.52	101.52 ± 2.40	108.70 ± 9.62	35.88 ± 4.76	39.82 ± 4.87	34.98 ± 1.75
Total vitamin A (n = 53)	156.38 ± 4.83	101.58 ± 2.20	108.90 ± 9.31	35.98 ± 4.76	39.90 ± 4.74	34.96 ± 1.71
Placebo						
BF (n = 6)	155.33 ± 6.34	100.17 ± 4.26	106.00 ± 14.03	34.17 ± 5.71	38.67 ± 7.94	34.83 ± 2.79
NF (n = 44)	156.14 ± 4.68	101.42 ± 2.40	107.25 ± 9.16	35.27 ± 4.73	39.02 ± 4.83	34.91 ± 1.51
Total placebo (n = 50)	156.04 ± 4.84	101.26 ± 2.67	107.10 ± 9.69	35.14 ± 4.81	38.98 ± 5.19	34.90 ± 1.67
Both treatment groups						
BF (n = 20)	156.50 ± 7.46	99.30 ± 9.15	108.00 ± 10.25	35.75 ± 5.02	40.00 ± 5.64	34.30 ± 3.29
BF (outlier excluded)	157.63 ± 5.64	101.26 ± 2.66	108.42 ± 10.35	35.63 ± 5.13	39.68 ± 5.61	34.89 ± 1.20
NF (n = 84)	155.89 ± 4.58	101.47 ± 2.38	107.94 ± 9.36	35.56 ± 4.72	39.40 ± 4.84	34.94 ± 1.61

[1] $\bar{x}$ ± SD. BSID II, Bayley Scales of Infant Development, second edition (20); MDI, mental development index; PDI, psychomotor development index; BRS, behavioral rating scale; BF, bulging fontanelle; NF, normal fontanelle.

TABLE 6
Classification of age-adjusted developmental index scores by treatment group and fontanelle status[1]

Score range: classification		BSID II reference population	Vitamin A		Placebo	
			Normal fontanelle	Bulging fontanelle	Normal fontanelle	Bulging fontanelle
MDI		%	(n) %		(n) %	
≥115:	accelerated	14.8	(25) 11.6	(9) 15.5	(31) 14.3	(5)15.2
85–114:	within normal limits	72.6	(186) 86.5	(46) 79.3	(180) 83.0	(26) 78.8
70–84:	mild delay	11.1	(3) 1.4	(2) 3.4	(5) 2.3	(2) 6.1
≤ 69:	significant delay	1.5	(1) 0.5	(1) 1.7	(1) 0.5	(0) –
PDI						
≥115:	accelerated	16.5	(49) 22.8	(16) 27.6	(47) 21.8	(5) 15.2
85–114:	within normal limits	68.7	(164) 76.3	(40) 69.0	(164) 75.9	(26) 78.8
70–84:	mild delay	12.5	(2) 0.9	(1) 1.7	(4) 1.8	(1) 3.0
≤69:	significant delay	2.3	(0) –	(1) 1.7	(1) 0.4	(1) 3.0

[1]BSID II, Bayley Scales of Infant Development, second edition (20); MDI, mental development index; PDI, psychomotor development index.

indicating that a bulging fontanelle modified the effect of vitamin A supplementation on linear growth. Compared with PL-NF children, and after adjusting for small baseline imbalances in maternal education and birth length, VA-BF children tended to grow 0.5 cm less (95% CI: −1.52, 0.52; $P = 0.33$) whereas VA-NF children grew 0.68 cm more (95% CI: 0.03, 1.33; $P < 0.05$) over the first 3 y of life. There was no difference in linear growth between PL-NF and PL-BF children ($P = 0.46$).

As shown in **Table 8**, height at 3 y was positively correlated with all developmental scores. To explore whether this association was likely to be due to differences in length at birth or to differences in growth over the first 3 y of life, correlation coefficients between developmental scores and birth length and between developmental scores and the residuals of birth length regressed on 3-y height are presented. Whereas birth length was weakly correlated with developmental outcome, the increment in stature over the first 3 y of life contributed most to the association between height and development.

DISCUSSION

This study provides no evidence that neonatal vitamin A supplementation, whether accompanied by a bulging fontanelle or not, is associated with biologically significant adverse developmental sequelae. Means and distributions of scores for 5 independent measures of development and for the total BRS score were not significantly different for children who received vitamin A or placebo nor for children within those treatment groups who did and did not have a bulging fontanelle. In addition, the distributions of scores for each treatment-fontanelle-specific group were within those of the reference population of US children used to standardize the BSID II. The strong associations in our data between developmental scores and maternal education and gestational age are consistent with the preponderance of previous work (27, 28), enhancing the validity of our findings. Our data are likely to have prognostic value. Whereas tests of mental function during infancy have almost no predictive power of later intelligence (29, 30), by 3 y of age, when our population was tested, there is a major gain in predictive power of school-age intelligence quotient (30). Our findings agree with those of a smaller study among Bangladeshi children in which no difference in development at 3 y of age was observed among children who developed a bulging fontanelle after receiving 50 000 IU vitamin A given simultaneously with diphtheria-pertussis-tetanus vaccine compared with vitamin A–supplemented and placebo-treated children who had a normal fontanelle (31).

Compared with PL-NF children, we observed a small (NS) 0.56-cm deficit in linear growth in VA-BF children and a small but significant 0.68-cm increment in length in VA-NF children. It may be that the vitamin A–induced bulging fontanelle caused this deficit in linear growth. If so, a 0.50-cm deficit in linear growth over the first 3 y of life among ≈3% of supplemented children may be a risk of this intervention. However, the magnitude of both the length deficit in VA-BF children and the length increment in VA-NF children was small and neither is likely to be of biological significance. At 3 y of age, 1 SD in height-for-age for a boy is 3.8 cm. Therefore, these length differences represent z scores of only −0.13 and 0.18.

Improvements or beneficial effects on development at 3 y of age were never expected outcomes of giving a single dose of vitamin A at birth. However, these data suggest that neonatal vitamin A supplementation may result in subtle improvements in development rate, especially in certain behavioral aspects. Whether these small benefits were mediated by a reduction in severe morbidity, indicated by the observed reduction in clinic visits for cough and fever (7), or by some direct effect of vitamin A on development is not known. Although small in magnitude, the parameter estimate for the effect of vitamin A on development was positive for all outcomes and was statistically significant in 1 of the 5 independent models and in 2 of these 5 models when 1 outlier was excluded. The magnitude of these benefits may again not be clinically or biologically significant. However, that this subtle benefit was strongest for 1 of the behavioral subscales (orientation-engagement) is of interest for 2 reasons. First, Horowitz (32) suggested that broad aspects of universal behaviors, such as those measured by the MDI and PDI, are unlikely to be easily influenced by nutritional status or other biological risk factors. Instead, less universally "grounded" behaviors, such as energy level, displays of happiness or pleasure, and enthusiasm (all assessed in the orientation-engagement factor), are more likely to be influenced by nutrition and other environmental factors. Second, a growing body of literature suggests that some behavioral factors are more predictive of later intelligence quotient than are tests of mental cognition (33–35). "Untestability," or behavior that is so problematic that the child is unable to complete the test and be scored, is perhaps the extreme example of this link between personality traits and intelligence. Bathurst and Gottfried (35) reported that developmental scores of 6-y-old

TABLE 7
Simple linear regression model for each developmental score: model A includes treatment and fontanelle status only; model B includes treatment, fontanelle status,and other baseline factors; model C is model B analysis after removal of outlier[1]

Covariate	Regression coefficient	P
Raw MDI A		
Treatment	0.41	0.37
Fontanelle status	−0.42	0.49
Raw MDI B		
Treatment	0.48	0.29
Fontanelle status	−0.32	0.60
Maternal education	0.33	0.0001
Raw MDI C		
Treatment	0.54	0.22
Fontanelle status	−0.08	0.89
Maternal education	0.32	0.0001
Raw PDI A		
Treatment	0.25	0.34
Fontanelle status	−0.72	0.04
Raw PDI B		
Treatment	0.27	0.30
Fontanelle status	−0.69	0.05
Maternal education	0.15	0.0001
Gestational age	0.40	0.01
Raw PDI C		
Treatment	0.39	0.07
Fontanelle status	−0.26	0.35
Maternal education	0.13	0.001
Gestational age	0.25	0.02
Total BRS A		
Treatment	1.09	0.14
Fontanelle status	−0.74	0.45
Total BRS B		
Treatment	1.20	0.10
Fontanelle status	−0.59	0.54
Maternal education	0.52	<0.0001
Gestational age	0.75	0.04
Orientation-engagement factor A		
Treatment	0.69	0.06
Fontanelle status	−0.27	0.58
Orientation-engagement factor B		
Treatment	0.74	0.04
Fontanelle status	−0.20	0.67
Maternal education	0.29	<0.0001
Gestational age	0.34	0.06
Emotional regulation factor A		
Treatment	0.21	0.59
Fontanelle status	−0.30	0.57
Emotional regulation factor B		
Treatment	0.23	0.56
Fontanelle status	−0.20	0.71
Maternal education	0.14	0.01
Male	−1.04	0.01
Motor quality factor A		
Treatment	0.22	0.14
Fontanelle status	−0.23	0.25
Motor quality factor B		
Treatment	0.24	0.10
Fontanelle status	−0.20	0.31
Maternal education	0.11	<0.0001
Gestational age	0.17	<0.03
Motor quality factor C		
Treatment	0.28	0.05
Fontanelle status	−0.07	0.70
Maternal education	0.11	0.001
Gestational age	0.12	0.09

[1] Treatment was coded as 0 = placebo, 1 = vitamin A; fontanelle status was coded as 0 = normal, 1 = bulging; male was coded as 0 = female, 1 = male; maternal education is in years; gestational age is in weeks. MDI, mental development index; PDI, psychomotor development index; BRS, behavioral rating scale.

TABLE 8
Pearson's correlation coefficient between developmental outcomes at 3 y of age with height at 3 y, length at birth, and the residuals of birth length regressed on 3 y height[1]

Developmental score	Height (3 y)	Birth length	Residual birth length on 3-y height
Raw MDI	0.18[2]	0.10[3]	0.17[4]
Raw PDI	0.22[2]	0.15[4]	0.20[4]
Total BRS	0.22[2]	0.14[4]	0.21[4]
Orientation-engagement	0.21[2]	0.12[4]	0.19[4]
Emotional regulation	0.11[4]	0.06	0.09[3]
Motor quality	0.33[2]	0.23[4]	0.30[4]

[1] MDI, mental development index; PDI, psychomotor development index; BRS, behavioral rating scale.
[2] $P < 0.001$.
[3] $P < 0.05$.
[4] $P < 0.01$.

children who had been "untestable" at 30–42 mo of age were significantly lower for a wide range of abilities than scores for children who were able to be tested. In this regard, it is of interest that the 3 children who were untestable in our study population were in the PL-NF group. These missing data may have biased our findings, weakening the benefit of vitamin A and strengthening the adverse effect of a bulging fontanelle.

It is important to consider these data in the context of the other findings of the trial. In this population, neonatal vitamin A supplementation reduced health care visits for signs and symptoms of pneumonia by > 50% and mortality by > 60% among infants during the first 4 mo of life. Risk of mortality during these early months is far greater than at any other time during childhood, yet is not amenable to common child survival interventions (eg, oral rehydration or immunizations). If the Indonesian results are replicated in studies currently underway in other settings, neonatal vitamin A supplementation could be promoted as an intervention to reduce morbidity and mortality among young infants throughout the developing world in a manner that is inexpensive and readily linked to existing health care systems. Data presented in this paper provide no evidence of any biologically significant risk associated with this intervention.

REFERENCES

1. Humphrey JH, West KP Jr, Sommer A. Vitamin A deficiency and attributable mortality among under-5-year-olds. Bull World Health Organ 1992;70:225–32.
2. Florentino RF, Tanchoco CC, Ramos AC, et al. Tolerance of preschoolers to two dosage strengths of vitamin A preparation. Am J Clin Nutr 1990;52:694–700.
3. Beaton GH, Martorell R, Aronson KJ, et al. Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. Geneva: Administrative Committee on Coordination/Sub-Committee on Nutrition, 1993. (ACC/SCN State of the Art Series Nutrition Policy Discussion Paper no. 13.)
4. Sugana T, Sommer A. Prevention of neonatal blindness. Lancet 1978;2:1101 (letter).
5. Sommer A. Vitamin A deficiency and its consequences: a field guide to their detection and control. 3rd ed. Geneva: World Health Organization, 1995.
6. West KP Jr, Katz J, Shrestha SR, et al. Mortality of infants < 6 mo of age supplemented with vitamin A: a randomized, double-masked trial in Nepal. Am J Clin Nutr 1995;62:143–8.

7. Humphrey JH, Agoestina T, Wu L, et al. Impact of neonatal vitamin A supplementation on infant morbidity and mortality rates. J Pediatr 1996;128:489–96.
8. Agoestina T, Humphrey JH, Taylor GA, et al. Safety of one 52-μmol (50,000 IU) oral dose of vitamin A administered to neonates. Bull World Health Organ 1994;72:859–68.
9. West KP Jr, Khatry SK, LeClerq SC, et al. Tolerance of young infants to a single, large dose of vitamin A: a randomized community trial in Nepal. Bull World Health Organ 1992;70:733–9.
10. Akib A, Muhilal, Munasir Z, et al. Clinical trial of vitamin A supplementation and the expanded programme on immunization. In: Proceedings of the XVI IVACG Meeting, 24–28 October, 1994, Chaing Rai, Thailand. Washington, DC: International Life Sciences Institute Research Foundation, 1994.
11. Baqui AH, de Francisco A, Arifeen SE, Siddique AK, Sack RB. Bulging fontanelle after supplementation with 25,000 IU vitamin A in infancy using EPI contacts. In: Proceedings of the XVI IVACG Meeting, 24–28 October, 1994, Chaing Rai, Thailand. Washington, DC: International Life Sciences Institute Research Foundation, 1994.
12. Stabell C, Balé C, Pedro da Silva A, Olsen J, Aaby P. No evidence of fontanelle-bulging episodes after vitamin A supplementation of 6- and 9-month-old infants in Guinea Bissau. Eur J Clin Nutr 1995;49:73–4.
13. de Francisco A, Chakraborty J, Chowdhury HR, et al. Acute toxicity of vitamin A given with vaccines in infancy. Lancet 1993; 342:526–7.
14. Sommer A, West KP Jr. Vitamin A deficiency: health, survival and vision. New York: Oxford University Press, 1996.
15. Ray J. Vitamin A supplementation in developing countries. Arch Dis Child 1995;73:275 (letter).
16. Seibert JJ, McCowan TC, Chadduck WM, et al. Duplex pulsed Doppler US versus intracranial pressure in the neonate: clinical and experimental studies. Radiology 1989;171:155–9.
17. de Francisco A, Hall AJ, Armstrong-Schellenberg JRM, Greenwood AM, Greenwood BM. The pattern of infant and childhood mortality in Upper River Division, The Gambia. Ann Trop Paediatr 1993; 13:345–52.
18. Fauveau V, Stewart MK, Chakraborty J, Khan SA. Impact on mortality of a community-based programme to control acute lower respiratory tract infections. Bull World Health Organ 1992;70:109–16.
19. Pandey MR, Daulaire NMP, Starbuck ES, Houston RM, McPherson K. Reduction in total under-five mortality in western Nepal through community-based antimicrobial treatment of pneumonia. Lancet 1991;338:993–7.
20. Bayley N. Bayley scales of infant development. 2nd ed. San Antonio, TX: The Psychological Corporation, 1993.
21. Idjradinata P, Pollitt E. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. Lancet 1993;341:1–4.
22. Husaini MA, Karyadi L, Husani YK, Sandjaja, Karyadi D, Pollitt E. Developmental effects of short-term supplementary feeding in nutritionally-at-risk Indonesian infants. Am J Clin Nutr 1991; 54:799–804.
23. Colleta NC, Satoto, Sockaling-ham S, Zeitlin M. The child development milestone chart—an approach to low cost programming in Indonesia. Early Child Dev Care 1993;96:161–71.
24. Wiklund L-M, Uvebrant P, Flodmark O. Computed tomography as an adjunct in etiological analysis of hemiplegic cerebral palsy; II: children born at term. Neuropediatrics 1991;22:121–8.
25. National Center for Health Statistics. NCHS growth curves for children: birth-18 years, United States. Hyattsville, MD: National Center for Health Statistics, 1977. [DHEW publication no. (PHS) 78:1650.]
26. Lubchenco LO, Hansman C, Boyd E. Intrauterine growth in length and head circumference as estimated from live births at gestational ages from 26 to 42 weeks. Pediatrics 1966;37:403–8.
27. McCall RB. Issues in the early development of intelligence and its assessment. In: Lewis M, Taft LT, eds. Developmental disabilities: theory assessment and intervention. New York: SP Medical and Scientific Books, 1981:177–84.
28. Aylward GP, Pfeiffer SI, Wright A, Verhulot SJ. Outcome studies of low birth weight infants published in the last decade: a meta analysis. J Pediatr 1989;115:515–20.
29. Kopp CB, McCall RB. Stability and instability in mental performance among normal, at-risk, and handicapped infants and children. In: Baltes PB, Brim OG, eds. Lifespan development and behavior. New York: Academic Press, 1980:33–61.
30. Wilson RS. The St. Louis Twin Study: developmental synchronies in behavior. Child Dev 1983;54:298–316.
31. van Dillen J, de Francisco A, Overweg-Plandsoen WCG. Long-term effect of vitamin A with vaccines. Lancet 1996;347:1705 (letter).
32. Horowitz FD. Using developmental theory to guide the search for the effects of biological risk factors on the development of children. Am J Clin Nutr 1989;50:589–97.
33. Birns B, Golden M. Prediction of intellectual performance at 3 years from infant tests and personality measures. Merrill-Palmer Quarterly 1972;18:53–8.
34. Bornstein MH, Sigman MD. Continuity in mental development from infancy. Child Dev 1986;57:251–74.
35. Bathurst K, Gottfried AW. Untestable subjects in child development research. Child Dev 1987;58:1135–44.

The American Journal of Clinical Nutrition

Official Journal of The American Society for Clinical Nutrition

9650 Rockville Pike, Bethesda, MD 20814-3998
(301) 530-7038 Fax (301) 571-8303
http://www.faseb.org/ajcn

ORIGINAL ARTICLE J. Nep Med Assoc 1998; 37:427-433

MATERNAL NIGHT BLINDNESS IN NEPAL : HEALTH RISKS AND CONSEQUENCES

Khatry SK[2] Christian P[1] Shrestha SB[2] Shrestha SR[2]
Kimbrough-Pradhan E[1] LeClerq SC[1] Pokhrel RP[2] West KP, Jr[1]

ABSTRACT :

Maternal night blindness is a problem of public health significance in vitamin A deficient areas of the world. In South and South-east Asia the condition is widely prevalent, especially during pregnancy. In Nepal, 10-20% of women during pregnancy and 5% of women during lactation experience night blindness. Two studies from the terai of Nepal provide insight into the risk factors and consequences of night blindness occuring in pregnancy. Women in Sarlahi consider "ratandho" (night-blindness) to be an important and severe illness of pregnancy for which they may seek treatment. It also adversely affects their day to day work activities and increases their reliance on other family members. Pregnant night blind women are vitamin A-deficient, reflected by a 4-fold higher risk of low serum retinol level (<20μg/dl) in these women compared to normal pregnant women. Night blind women are also likely to be wasted, ill, and anemic than women without night blindness. Women who suffered from night blindness during pregnancy were at a higher risk of dying in the subsequent two year period than women who did not experience night blindness. We recommend that night blindness be used as a simple tool to identify high risk women during pregnancy for antenatal care and nutritional intervention.

Ker words : night blindness, pregnancy, Sarlahi, vitamion A deficiency.

INTRODUCTION :

In developing countries where vitamin A deficiency is endemic, night blindness is one of the first clinical symptoms to occur in preschool age child populations. Night blindness is caused by an inability of the rod cells in the retina of the eye, due to an inadequacy of vitmin A, to generate a photosensitive pigment called rhodopsin, which is required

1 *Center for Human Nutrition, Department of International Health, Johns Hopkins School of Hygiene and Public Health, 615 N. Wolfe St. Baltimore, MD., USA.*
2 *National Society for the Prevention of Blindness (Nepal Netra Jyoti Sangh), P.O. Box 335, Kathmandu, Nepal.*

62

to see in dim illumination. This condition, while common in children, has received little attention among adults. In the 1960s night blindness was found among pregnant women in India presenting at clinics, which, in some cases, responded to treatment with high doses of vitamin A. Night blindness occuring during pregnancy is unique as it manifests in the latter half of pregnancy and disappears spontaneously after delivery, although it may reappear in some instances during lactation. Recently, research in Nepal has brought this problem to the forefront. Several reports of the prevalence of night blindness have emerged from surveys conducted in different regions of Nepal that range from 8% to 52% during pregnancy and 5-6% during lactation[3,4] Linehan et al 1996). The national average estimate of night blindness prevalence according to a recent demographis health survey is 18% although its prevalence varies substantially by district.

This paper describes the findings of two studies on night blindness carried out in Sarlahi district in the southern terai region of Nepal. The first was an ethnographic research study investigating the communities' perceptions of its importance, causes, and treatments. The second was a case-control study designed to examine the nutritional and health-related risk factors of night blindness during pregnancy.

I. ETHNOGRAPHIC STUDY

Materials and Methods :

The purpose of this study was to identify local terms and concepts of maternal night blindness, investigate women's perceptions of its causes, and descriptions of its treatment and consequences.Tthe study was conducted in three village development committee (VDCs) of Sarlahi district during September through November of 1993. Qualitative research methods including in-depth interviews, free-listing, and quick-sort ranking were used. In all 38 interviews were conducted with pregnant women (some of whom had a curent or previous history of night blindness), lactating women, traditional birth attendants, and women with children. The interviews provided case-histories of night blindness and pregnancy naratives which covered domains of women's illness, diet, beliefs related to pregnancy and lactation, and treatment seeking behavior. Free-listing was done using a standard question to obtain a list of women's illnesses (n=25)[8]. these same women were asked to rank 15 of the most commonly mentioned women's illnesses in order of perceived severity[9].

Results :

The term used for night blindness in the three languages spoken in this part of the terai were "ratauni" in Maithili, "ratandho" in Nepali and "rataunji" in Tharu. although a majority of women understood these terms, some women described the symptoms of the condition such as "poor vision at night" when referring to it. Other descriptions of the symptoms were "smoke in the eyes" and "haziness". The onset of night blindness was mostly in the late second or third trimester. Most women reported that it usually disappeared soon after delivery, although some said it could recur during lactation. A majority of the women believed night blindness to be associated with pregnancy and attributed 'pregnancy" to be its cause.Tthis is suggested from a quote from a maithili woman. She said: *"Don't know what causes it ... it happens to some women who are pregnant, like the swelling of hands and feet. It happens on its own, because of the pregnancy."* "Kamjori" (weakness) and "Garmi" (hotness) were also mentioned frequently as causes of night blindness in women. Women tended not to seek any

63

treatment for night blindness, although some mentioned treating it with vitamin A "goti" (pills), available cheaply at local pharmacies and shops. The most common pills were small, round and red in color with 5,000 IU of vitamin A and IU of vitamin D. These were popularly known as "ratandho goti" although none of the women appeared to know that they contained vitamin A.

Night blindness was ranked second (to vaginal bleeding) in perceived severity among the 15 most frequently mentioned illnesses by women. This ranking suggests that women considered it a serious illness. Apart from this, night blindness was perceived to impact on women's night time work activities. Direct observations of two women suffering from night blindness at night revealed that while women could work in the light of a fire, they were rendered inactive when they moved away from this source of light. women reported relying on other family members for various activities such as child care and cooking, as well as having to curb outdoor activities such as fetching water in the evening. They also described difficulty in eating, accidents and other problems such as the social stigma attached to being night blind.

Women did not draw a direct association between food intake and night blindness, although a couple of women mentioned eating "cold" foods to counter-act the hotness of the body which they believed caused night blindness. However, food-related beliefs and practices of pregnancy are important to understand the relationshiop between diet and night blindness during pregnancy. With the research we were able to provide an understanding of the cultural context within which night blindness occurs during pregnancy, and its saliency in the lives of women living in rural southern Nepal.

II. CASE-CONTROL STUDY

Materials and Methods :

Subsequent to the ethnographic study, a population based, case-control study of night blindness during pregnancy was carried out to explore the risk factors associated with this condition (christian et al 1998). This study was conducted in 19 of the 30 VDCs of Sarlahi district where the Nepal Nutrition Intervention Project-Sarlahi (NNIPS) was underway, supplementing women of reproductive age with vitamins. Local women distributed the supplements to women in their area and identified those who became pregnant. The local distributore were trained to obtain night blindness histories using local terms for the condition from all pregnant women in the community they served. Pregnant women were asked "*tapailai ahile ratandho bhaeko cha ?*" (or the appropriate equivalent in Maithili/Tharu) every week from the time they became pregnant until the termination of the pregnancy. Women reporting night blindness were verified by a team of data collectors who were trained to interview women about their symptoms and the impact night blindness had on their activities. Women who reported poor vision during daytime were considerd to be "false" cases and were not enrolled in the study. Over a period of 15 months, 117 cases were identified and enrolled in the study. The same number of controls were randomly selected from the pool of women who were pregnant but did not have night blindness. The controls were matched with the cases for their gestational month of pregnancy. After selection, cases and controls were visited in their homes within a week for an interview and an examination. Data were collected on women's previous 7-day frequency of intake of certain vitamin A-rich foods, morbidity history, and household socioeconomic characteristics. Capillary blood was drawn by finger stick for serum

64

retinol, β-carotene, and hemoglobin (Hb) estimation. anthropometric measurements included weight, height, mid-upper arm circumference (MUAC) and triceps skinfolds (TSF)[10].

Results :

While cases and controls were similar with respect to age and the number of previous pregnancies, cases had lower socioeconomic status, were less likely to be literate, and belonged to a lower caste than controls. The mean gestational age of cases and controls was 7 months.

The following table shows the differences in various nutritional status indicators between cases and controls.

	Case Mean	Control Mean	Mean difference (SE)
Serum retinol (μg/d)	20.6	29.4	-8.6* (1.42)
Hb (g/d)	8.9	9.6	-0.7* (0.22)
Weight (kg)	45.2	47.7	-2.6* (0.63)
Height (cm)	149.6	151.0	-1.3 (0.72)
MUAC (cm)	21.8	22.7	-0.9*)0.23)
BMI (wt/ht^2)	20.2	20.9	-0.8* (0.22)
TSF (mm^2)	8.7	9.5	-0.8* (0.34)

* $p < 0.05$

These data clearly show that cases were exposed to an excess risk of vitamin A deficiency compared to controls. At the same time in pregnancy, cases had -9 μg/dl lower serum retinol and were 4 times more likely (95%) confidence interval 2.2 - 7.4) to have low serum retinol levels (<20 μg/dl) than controls. Hb levels were also significantly ($p<0.05$) lower among cases (by 0.7 g/dl) and were three times more likely to be < 7g/dl (cut-off for severe anaemia) than controls. women with night blindness had significantly ($p<0.05$) lower body weight, MUAC, bosy mass index (BM) and TSF than controls, suggesting that protein-energy malnutrition could contribute to night blindness.

Cases were 2-3 times likely to have consumed preformed and carotenoid sources of vitamin A inhhte past week than the controls in the summer months of April through September (Figure 1). This difference was stastically significant for all four food groups examined. In winter, lasting from October through March (figure (2), however, only milk and milk products (whey and cream) and carotenoid sources (dark green leafy vegetables and mango) tended to be consumed less by cases than controls, whereas meat, eggs, and fish were equally likely to be eaten by cases and controls. This seasonal variation in the risk of inadequate intake of vitamin A foods suggests that targeting women during a season of low food availability and access may be an effective strategy to adopt for nutrition intervention programs.

Seven-day morbidity histories were obtained at the time of the night blindness episode among cases and at the same time in gestation among controls. Cases were 2-3 times more likely to report symptoms of urinary/reproductive tract infections such as lower abdominal pain, painful urination, and vaginal discharge (Figure 3). Cases were also significantly more likely to report diarrhoea as well as symptoms of upper gastrointestinal infection such as nausea, vomiting, and low appetite in the previous week. Although not presented here, our data also show that an acute phase response following an infection may be strongly associated with hyporetinemia in these women, especially night blind women.

65

Figure 1. OR (95% CI) of eating vitamin A foods in the past 7 days among cases relative to controls - SUMMER

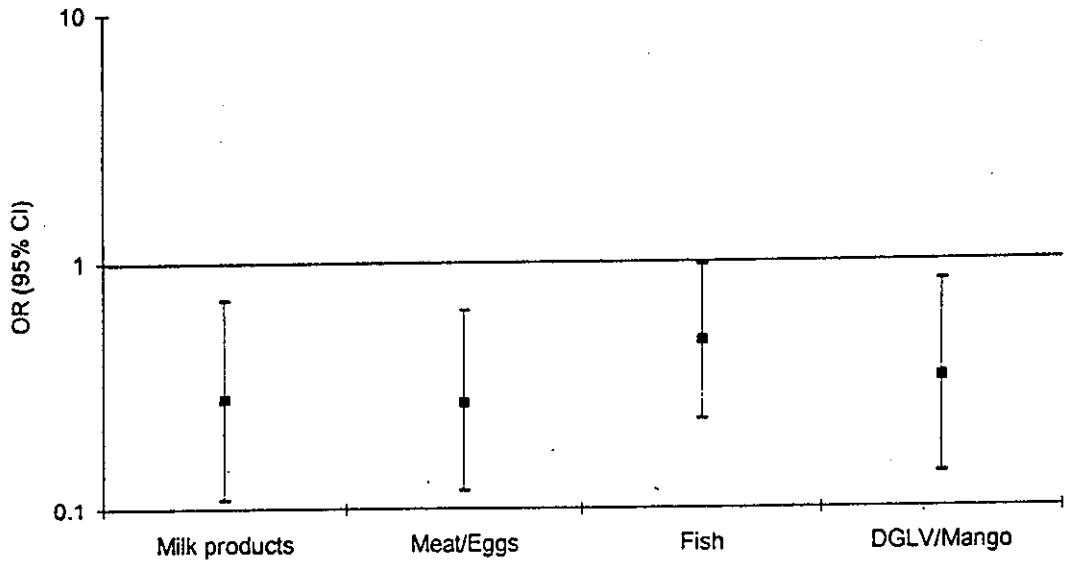
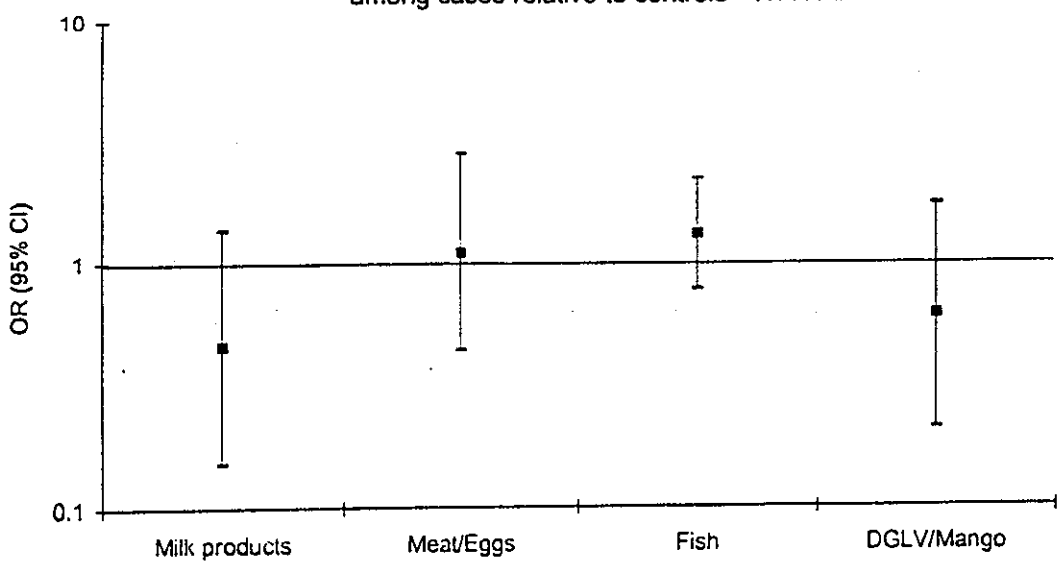
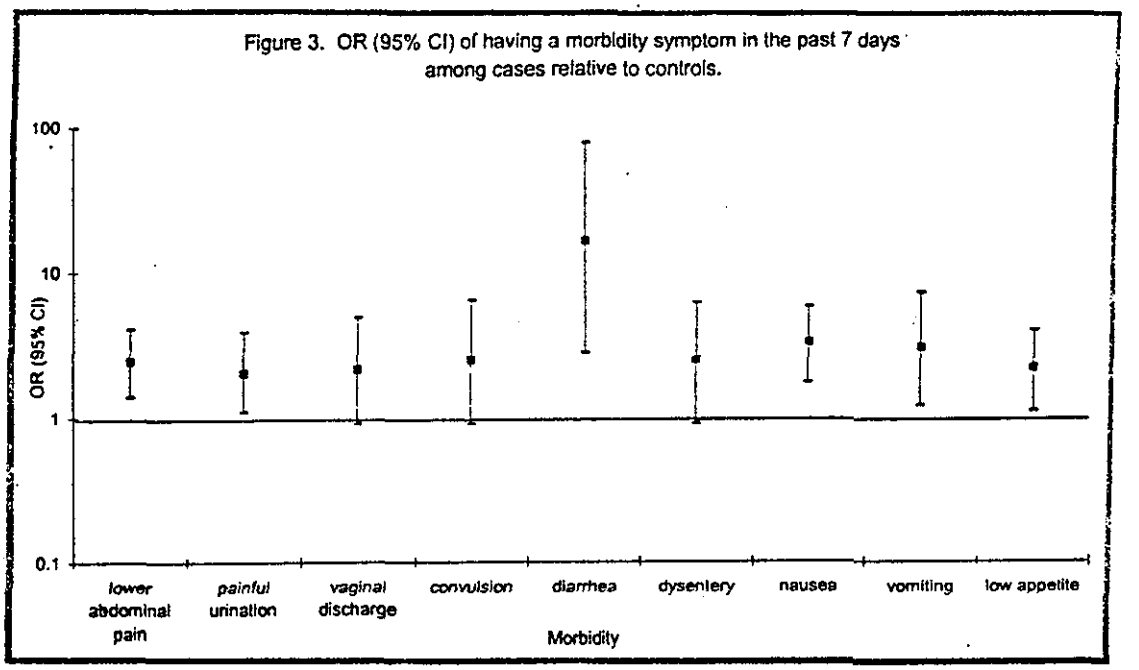


Figure 2. OR (95% CI) of eating vitamin A foods in the past 7 days among cases relative to controls - WI NTER



66



DISCUSSION :

Night blindness occurs commonly in the latter half of pregnancy, affecting 18% of pregnant women nationwide. Night blind pregnant women in rural Nepal are exposed to not only a risk of vitamin A deficiency, a known determinant of the condition, but also other risks including anaemia, protein energy malnutrition, and infectious and non-infectious illnesses. Preliminary analysis suggests that unintervened women who reported night blindness during pregnancy were at a 7-fold night blindness[8]. A simple history of maternal night blindness may help to identify high-risk pregnancies and should be included in antenatal assessment and care programs. *symptoms of blurred or hazy vision at dusk* with adequate vision during the daytime may be used to verify the history. Night blindness is perceived to be an important illness of pregnancy which may affect a woman's role as a caretaker and mother and impact her day to day actively patern. This condition, which has not received sufficient attention previously, should be routinely investigated to further define its extent and to develop effective food-based and other *strategies for its prevention*. Night blindness history can serve as a simple tool to identify high risk women for antenatal and postnatal care.

ACKNOWLEDGEMENTS :

We thank members of the Nepal Nutrition Intervention Project-Sarlahi in Nepal for their assistance in the conduct of the study, especially DN mandal, TR Shakya, I. Shrestha, K Dhakal, G subedi, U Shankar, A Bhetwal, DB Khadka, the area coordinators, team leader/interviewers and the 300 ward distributors without whom the study would not have been possible. This study was supported by the Office of Health and Nutrition, U.S. Agency for International Development (USAID) and assisted by Task Force Sight and Life, Bases, Switzerland and the Sushil Kedia Foundation, Hariaun, Sarlahi, Nepal.

REFERENCES :

1. Mandal GS, Nanda KN, Bose J. Night blindness in pregnancy. J. Obstet Gynecol India 1969; 19: 453-458.

2. Dixit DT. Night blindness in third trimester of pregnancy. Indian J Med Res 1966; 54: 791-5.

3. Starbuck ES. Night blindness during pregnancy as a risk marker for early infant death in Jumla, Nepal (dissertation). Johns Hopkins University, Baltimore, maryland, 1993.

4. Katz J, Khatry SK, West KP Jr., Humphry J, LeClerq SC, Kimbrough-Pradhan E, Pokhrel RP, sommer A. Night blindness is prevalent during pregnancy and lactation in rural Nepal. J Nutr 1995; 125: 2122-7.

5. Linehan M, shrestha A, Ban B, Rycus P. Xerophthalmia p[revalence in ten districts of the far and mid-easter regions of Nepal. Report of the XVII Internationa Vitamin A consultative Group meeting. Guatemala city, guatemala. 18-22 March 1996. International Life Sciences Institute, Washington, DC 1996.

6. Christian P, Bently ME, Pradhan R, West KP Jr. an ethnographic study of night blindness (ratauni) among women in the terai of Nepal. Soc Sci Med 1997; (inn press).

7. Christian P, West KP Jr., Khatry SK, Karz J, Shrestha SR, Pradhan EK, LeClerq SC, Pokhrel RP. Night blindness of pregnancy in rural Nepal - nutritional and health risks. Int J Epi 1998 (in press).

8. Bernard HR. Research methods in Anthropology. Qualitative and Quantitative Approaches. 2nd ed. Delhi, Sage Publications Inc. 1994.

9. Weller SC and Romney KA. Systematic DAta Collection. Qualitative Research Methods vol 10, Delhi, SAge Publication Inc. 1988.

10. Gibson RS. principles of nutritional assessment. New York, Oxford University Press 1990.

68

Maternal Vitamin A Deficiency and Infant Mortality in Malawi

by Richard D. Semba* MD, MPH, Paolo G. Miotti** MD, MPH, John D. Chiphangwi*** MD, Gina Dallabetta** MD, MPH, Li-Ping Yang** MA, Alfred Saah** MD, MPH, *and* Donald Hoover** MPH, PhD

**Department of Ophthalmology, School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA*

***Department of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland, USA*

****School of Medicine, University of Malawi, Blantyre, Malawi*

Summary

The relationship between maternal vitamin A deficiency during pregnancy and infant mortality is unclear. We conducted a prospective cohort study of 377 HIV-negative women and their infants in Blantyre, Malawi. Serum vitamin A levels were measured during the second or third trimester of pregnancy and infants were followed during the first year of life. From delivery until 12 months of age, 18 infants died (47.7 per 1000). Mothers of infants who died had lower serum vitamin A levels during pregnancy ($0.74 \pm 0.13\,\mu mol/l$) compared with mothers of infants who did not die ($1.02 \pm 0.03\,\mu mol/l$) ($p = 0.055$). Infants born to women whose vitamin A levels were in the lowest quartile ($< 0.32\,\mu mol/l$) had three-fold higher likelihood of mortality than infants born to women whose vitamin A levels were in the higher quartiles ($p < 0.03$). These results suggest that maternal vitamin A deficiency during pregnancy may contribute to higher infant mortality rates.

Introduction

Vitamin A is an essential micronutrient for immunity, growth, reproduction, cellular differentiation, maintenance of epithelial surfaces, and vision. In developing countries, vitamin A deficiency is a leading cause of morbidity, mortality, and blindness among children.[1] Pregnant women are at a higher risk of developing vitamin A deficiency because of an increased demand for vitamin A by the developing fetus. Although animal studies demonstrate that vitamin A deficiency during pregnancy is associated with reproductive failure and high rates of mortality of offspring,[2,3] the situation in humans is less clear. Recently we showed that vitamin A deficiency during pregnancy in human immunodeficiency virus (HIV)-infected women is associated with increased mother-to-child transmission of HIV and higher infant mortality rates.[4,5] In individuals without HIV infection, it is unknown whether vitamin A deficiency during pregnancy is related to infant mortality. We addressed this question within the context of a longitudinal cohort study of non-HIV-infected women and their infants in Malawai.

Acknowledgements

This study was supported in part by the National Institutes of Health (AI26499, AI33874, HD30042, HD32247) and the United States Agency for International Development (Cooperative Agreement DAN-0045-A-5094-00). The authors acknowledge the dedication of the staff of the Johns Hopkins/University of Malawi project, the Malawi Health Sciences Committee, and the Ministry of Health for this continuing productive collaboration. The authors acknowledge the technical assistance of Yin Lan in measurements of serum vitamin A levels.

Correspondence: Dr Richard D. Semba, Ocular Immunology Service, Suite 700, 550 North Broadway, Baltimore, MD 21205, USA. Tel. 00 1 410 955 3572; Fax 00 1 410 955 0629.

Subjects and Methods

The study population consisted of 377 mothers and their infants who were born at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, from November 1989 to August 1991. Between October 1989 and August 1991, women attending the antenatal clinic of QECH were screened for HIV-1 infection using an enzyme-linked immunosorbent assay (ELISA) (Wellcozyme; Wellcome Diagnostics, Dartford, UK). Pregnant women who were HIV seropositive were excluded from the study. Age, weight, and height of the mothers were recorded at the first prenatal visit, which was during the second or third trimester of pregnancy. Maternal body mass index[5] was calculated as follows: weight, kg/(height, m)^2. Serum samples were obtained at the first prenatal visit, stored at −70°C, and analysed for vitamin A by high performance liquid chromatography (HPLC).[5] Detailed information was obtained regarding socioeconomic status of the study subjects including level of education of both partners, occupation, housing, and basic amenities like running water and electricity at home.

 © Oxford University Press 1998

69

At delivery, gestational age was estimated by Ballard score and the infant's weight was recorded. Mothers and their infants were followed every 3 months after delivery with a history and physical examination and vital events were known for all infants until 12 months of age. At each follow-up visit, mothers were asked whether they had stopped breastfeeding. Studies were approved by the Malawi Health Sciences Committee (Blantyre) and the Committee on Human Research at Johns Hopkins University (Baltimore).

In the statistical analysis, means (± SE) were reported and comparisons made with the Wilcoxon rank sum test. For categorical variables, proportions are reported and univariate comparisons were made with contingency tables by using exact or trend tests of statistical significance. Rank correlations were performed. Continuous variables were also divided into quartiles for comparison of survival rates. Socioeconomic status was ruled out as a potential confounder with regard to infant mortality.

Results

Two-hundred and eleven of 377 pregnant women (55.9 per cent) had serum vitamin A levels consistent with vitamin A deficiency in adults (< 1.05 μmol/l). One-hundred and nineteen women (31.5 per cent) had serum vitamin A levels below 0.35 μmol/l. Eighteen infants died between delivery and 12 months of age (infant mortality rate of 47.7 per 1000). There were three sets of twins of which one twin died. The characteristics of infants who died and survived and their mothers are shown in Table 1. There were no differences in age and body mass index between mothers whose infants died or survived, but serum vitamin A levels were lower among mothers of the infants who died ($p = 0.055$). Infants who died were also of lower birthweight and gestational age. Of 359 mothers whose infants survived to 12 months of age, 355 (98.9 per cent) mothers reported that they were still breastfeeding their infants at 12 months of age, one mother had discontinued breastfeeding, and breastfeeding status was unknown for three mothers.

Mothers with and without serum vitamin A levels consistent with deficiency are compared in Table 2. There were no significant differences in maternal age or body mass index between the vitamin A-deficient and non-deficient mothers. There were no significant differences in birthweight, gestational age, or percentage of infants who died between vitamin A-deficient and non-deficient mothers. Because of the small numbers of deaths, we divided maternal vitamin A levels into quartiles to evaluate maternal vitamin A levels with respect to infant mortality rates. The 25th, 50th, and 75th

TABLE 1

Clinical status of mothers whose infants died or survived the first 12 months of life

Characteristic[a]	Infant died ($n = 18$)	Infant survived ($n = 259$)	p value
Mother			
Age (years)	24 (18, 24)	25 (20, 30)	0.97
Body mass index (wt/ht^2)	2.36 (2.26, 2.55)	2.35 (2.18, 2.50)	0.84
Vitamin A (μmol/l)	0.41 (0.31, 1.09)	0.96 (0.32, 1.44)	0.055
Infant			
Birthweight (g)	2750 (2600, 3200)	3000 (2800, 3300)	0.073
Gestational age (weeks)	38.5 (35.5, 40)	40 (38, 40)	0.042

[a] Median (interquartile range) for each characteristic.

TABLE 2

Clinical status of vitamin A-deficient and non-deficient mothers and their infants

Characteristic[a]	Vitamin A-deficient ($n = 211$)	Vitamin A normal ($n = 166$)	p value
Mother			
Age (years)	25 (20, 31)	24 (20, 29)	0.10
Body mass index (wt/ht^2)	2.35 (2.18, 2.53)	2.34 (2.16, 2.45)	0.44
Vitamin A (μmol/l)	0.32 (0.30, 0.78)	1.47 (1.25, 1.79)	0.0001
Infant			
Birthweight (g)	3000 (2800, 3240)	3000 (2800, 3300)	0.26
Gestational age (weeks)	40 (38, 40)	40 (38, 40)	0.47
Infants who died (%)	5.7	3.6	0.16

[a] Median (interquartile range) for each characteristic.

70

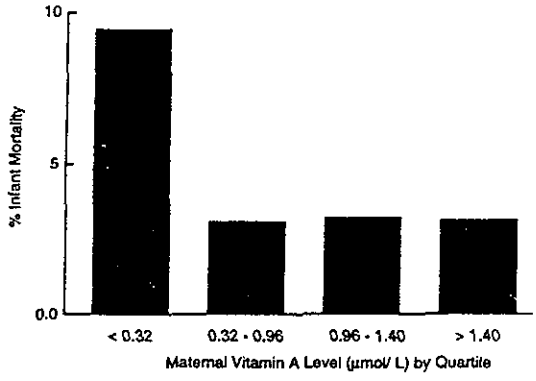


FIG. 1. Maternal vitamin A levels (by quartile) and infant mortality rates. Women whose vitamin A level was in the lowest quartile had higher infant mortality rate ($p < 0.03$).

percentiles of serum vitamin A levels were 0.32, 0.96, and 1.40 μmol/l. Infant mortality rate was significantly higher among those born to mothers whose vitamin A level was in the lowest quartile (Fig. 1) ($p < 0.03$).

Discussion

Vitamin A deficiency was common among pregnant women in this study population. Nearly one-third of the women had serum vitamin A levels < 0.35 μmol/l, a level at which hepatic reserves of vitamin A are likely to be depleted. Factors known to contribute to vitamin A deficiency in pregnant women include low intake of vitamin A-containing foods, increased demand for vitamin A by the fetus, haemodilution of pregnancy, and abnormal urinary losses of vitamin A in pregnancy.[6]

To our knowledge, this is the first study to show an association between maternal vitamin A deficiency during pregnancy and infant mortality in individuals without HIV infection. We previously showed that among pregnant women with HIV infection, maternal vitamin A deficiency during pregnancy was associated with increased infant mortality rates, but the HIV status of the infants who died was unknown.[5] It was unclear whether the infants died because they had HIV-related complications or had died from other causes. The present study in non-HIV-infected individuals suggests that this relationship between low maternal vitamin A levels and infant mortality also exists in populations without HIV infection. This observation is consistent with animal model studies which show increased mortality rates in offspring born to vitamin A-deficient animals.[2,3]

Vitamin A deficiency is a nutritional immunodeficiency disorder characterized by widespread alterations in mucosal surfaces, antibody responses, and cell-mediated immunity.[1,7] A potential biological mechanism which might explain the increased mortality among infants born to vitamin A-deficient mothers is immune impairment in the infants. Vitamin A deficiency in the mothers could possibly result in lower titres of protective antibodies being transferred passively to the infants, conferring lower protection to a wide variety of pathogens.[5]

The infant mortality rate in this study population was 47.7 per 1000 compared with a reported infant mortality rate of 141 per 1000 for Malawi.[8] The rate in our study was probably lower because the participants were receiving more intensive, regular medical care every 3 months. The sample size and the number of infant deaths were relatively small in this study. Although we did not observe a difference in infant birthweight between those born to vitamin A-deficient and non-deficient mothers, this could perhaps be due to limited statistical power of our sample size.

It is not known whether the low vitamin A levels are causally involved in the higher infant mortality rate or whether the low levels are a 'marker' for another, more important, factor. The present study is limited in that other micronutrients, such as iron, folate, and zinc, were not measured. However, among the micronutrients, vitamin A plays an important role in immune function,[1,7] and vitamin A has been known as the 'anti-infective vitamin' since the 1920s. It is unknown whether correction of maternal vitamin A deficiency during pregnancy will improve survival of infants. Clinical trials are currently in progress in the Asian subcontinent and in sub-Saharan Africa to determine whether antenatal vitamin A supplementation can reduce infant mortality rates.

References

1. Sommer A, West Jr KP. Vitamin A Deficiency: Health, Survival, and Vision. Oxford University Press, New York, 1996.
2. Mason KE. Foetal death, prolonged gestation, and difficult parturition in the rat as a result of vitamin A-deficiency. Am J Anat 1935; 57: 303–49.
3. Apgar J, Dulin A, Kramer T, Smith JC. Reduced survival of neonates due to vitamin A deficiency during pregnancy in the guinea pig. Proc Soc Exp Biol Med 1991; 197: 56–8.
4. Semba RD, Miotti P, Chiphangwi J, *et al.* Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. Lancet 1994; 343: 1593–7.
5. Semba RD, Miotti PG, Chiphangwi JD, *et al.* Infant mortality and maternal vitamin A deficiency during HIV infection. Clin Infect Dis 1995; 21: 966–72.
6. Gaehtgens G. Über die Ausscheidung von Vitamin A in der Gravität. Klin Wochenschr 1937; 16: 52–3.
7. Semba RD. Vitamin A, immunity, and infection. Clin Infect Dis 1994; 19: 489–99.
8. UNICEF. State of the World's Children 1995; Oxford University Press, New York, 1995.

71

The Role of Vitamin A and Related Retinoids in Immune Function

Richard D. Semba

Johns Hopkins University School of Medicine

Introduction

Vitamin A and related retinoids play an important role in the regulation of immune function. Vitamin A deficiency compromises immunity, resulting in major morbidity and mortality. The link between clinical vitamin A deficiency (nightblindness and xerophthalmia) and infectious disease morbidity and mortality has been known for hundreds of years. Experimental observations and clinical trials in the 1920s and 30s led to the reputation of vitamin A as the "anti-infective" vitamin (Green and Mellanby 1928). By the 1960s, it was noted that among the micronutrients, "no nutritional deficiency is more consistently synergistic with infectious disease than that of vitamin A" (Scrimshaw et al. 1968). More recent community- and hospital-based clinical trials show that vitamin A supplementation reduces child mortality by 20–30% (Beaton et al. 1993). Vitamin A capsule distribution is recognized as one of the most cost-effective interventions to improve health (World Bank 1993) and ranks among vaccination and oral rehydration therapy in importance as a public health measure.

The underlying basis for the use of vitamin A supplementation to reduce infectious disease morbidity and mortality is its role in enhancing immunity. Vitamin A and related retinoids have therapeutic potential as immune modulators, and some of these uses are also beginning to be realized in the fields of dermatology and oncology. Over the last several decades, major progress has been made in elucidating the role of vitamin A and related retinoids in immune function. The discovery of retinoic acid receptors has greatly facilitated our understanding of how vitamin A may influence immune function at the genetic level. Improvements in analytical methods have led to the discovery of new natural metabolites of vitamin A and retinoic acid, which suggests immense complexity in the regulation of biologic responses by retinoids. The purpose of this review is to provide an overview of the different effects that vitamin A and related retinoids play in immune function, with an emphasis on developments in the last decade (Table 1). Comprehensive coverage of the earlier literature can be found in previous reviews (Nauss 1986, Ross 1992, Semba 1994a).

Retinoids and Nuclear Receptors

The widespread immunologic effects of vitamin A appear to be mediated primarily through its acid derivatives, which include all-*trans* retinoic acid and 9-*cis* retinoic acid (Chytil and Haq 1990, Pfahl and Chytil 1996, Napoli 1996). There are numerous other natural isomers of retinoic acid that have been identified and may help explain the pleiotropic effects of retinoids (Pfahl and Chytil 1996). Two families of nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs), consist of three isotypes (α, β, γ) and control gene expression by retinoid signals (Chambon 1996). The expression of RAR and RXR isotypes varies in different tissues, and vitamin A deficiency may selectively alter the expression of certain isotypes in various tissues (Haq et al. 1991). All-*trans* retinoic acid is a ligand for RARs, whereas 9-*cis* retinoic acid is a ligand for both RARs and RXRs.

RARs and RXRs form RXR/RAR heterodimers and bind to retinoic acid response elements (RAREs) of target genes. Most RAREs have been identified in the regulatory region of a variety of genes. RXR may also form heterodimers with the thyroid hormone receptor, vitamin D_3 receptor, peroxisome proliferator activator receptors, and a number of newly described "orphan receptors." In the presence of 9-*cis* retinoic acid, RXR/RXR homodimers may form and recognize a subset of RXREs or inhibit the formation of certain heterodimers (Pfahl and Chytil 1996). Orphan receptors such as chicken ovalbumin up-stream promoter transcription factor (COUP-TF) (Tran et al. 1992), ARP-1, TAK1 (Hirose et al. 1995), RVR (Retnakaran et al. 1994), RZR (Carlberg et al. 1994), and thymus orphan receptor (TOR) (Ortiz et al. 1995) may repress or modulate the induction of genes by retinoic acid. Thus, RARs and RXRs may interact with multiple transcriptional mediators and/or corepressors, adding an enormous level of complexity to regulation of retinoic acid responses. Experiments with RAR-deficient, or "knock-out," mice suggest that there may be a great deal of functional redundancy involved in signaling (Kastner et al. 1995). Other vitamin A metabolites in the retroretinoid family may support biologic functions via a pathway that is distinct from the retinoic acid pathway. 14-Hydroxy-4,14-*retro* retinol sup-

72

ports, whereas anhydroretinol inhibits, cell growth (Buck et al. 1991, Buck et al. 1993, Eppinger et al. 1993). In addition, the oxoretinoids may play a role as retinoic acid receptor ligands (Blumberg et al. 1996).

Mucosal Immunity

Vitamin A deficiency compromises mucosal immunity by altering the integrity of mucosal epithelia, including those of the eye as well as the respiratory, gastrointestinal, and genitourinary tracts. In addition, secretory IgA (sIgA) responses to various pathogens may be impaired by vitamin A deficiency (Wiedermann et al. 1993a, Stephensen et al. 1996, Gangopadhyay et al. 1996). Ocular changes related to vitamin A deficiency include squamous metaplasia of the conjunctiva and cornea, loss of goblet cells, abnormal keratinization, and keratomalacia (Tseng et al. 1984, Sommer and West 1986). Lactoferrin, an iron-binding glycoprotein involved in immunity to bacteria, viruses, and fungi, is found in mucosal secretions, and its expression appears to be modulated by vitamin A. Tear lactoferrin levels are increased by vitamin A supplementation in children (Van Agtmaal 1989). Pathologic changes in the ocular surface may result in increased susceptibility to infection, for example, as shown in animal models of *Pseudomonas* keratitis (Twining et al. 1996a).

The normal tracheobronchial tree consists of ciliated columnar respiratory epithelium with mucus and goblet cells. The mucociliary escalator continuously traps and removes inhaled microorganisms and carcinogens and is an important first line of immune defense for the tracheobronchial tree. Vitamin A deficiency in rats and hamsters leads to loss of ciliated epithelial cells and mucus in the tracheobronchial tree and replacement by stratified, keratinized epithelium (Wilhelm 1954, Wong and Buck 1971, McDowell et al. 1984). Extensive squamous metaplasia of the respiratory tract was described in autopsy studies of vitamin A–deficient children (Blackfan and Wolbach 1933, Sweet and K'ang 1935). These pathologic alterations of the respiratory epithelium may partially explain the epidemiologic observation that children with mild vitamin A

Table 1. Immune Response Elements That May Be Regulated By Retinoids

Element	References
Keratinization	Fuchs and Green 1981, Eckert and Green 1984, Molloy and Laskin 1985, Molloy and Laskin 1988, Gijbels et al. 1992, Darwiche et al. 1993
Mucin production	DeLuca et al. 1969, Rojanapo et al. 1980, Manna et al. 1994, Manna et al. 1995
Hematopoiesis	Semba et al. 1993a, Semba et al. 1993b, Göttgens and Green 1995, Hussey et al. 1996
Apoptosis	Iwata et al. 1992, Yang et al. 1993, Nagy et al. 1995, Yang et al. 1995, Horn et al. 1996
Neutrophil function	Twining et al. 1996b, Twining et al. 1997
Natural killer cell function	Zhao et al. 1994a, Zhao et al. 1994b, Zhao and Ross 1995, Hussey et al. 1996
Monocyte/macrophage function	Breitman et al. 1980, Tachibana et al. 1984, Hemmi and Breitman 1985, Trechsel et al. 1985, Katz et al. 1987, Dillehay et al. 1988, Turpin et al. 1990, Hope et al. 1990, Matikainen et al. 1991, Guzman et al. 1991, Öberg et al. 1993, Szabo et al. 1994
Langerhans cell function	Maisey and Miller 1986, Katz et al. 1987b, Meunier et al. 1994, Sailstad et al. 1995
T lymphocyte function	Garbe et al. 1992, Friedman et al. 1993, Rahman et al. 1997
Cytotoxic T lymphocyte function	Malkovský et al. 1983a, Sijtsma et al. 1990
B lymphocyte function	Buck et al. 1990, Israel et al. 1991, Blomhoff et al. 1992, Wang et al. 1993a, Wang and Ballow 1993b, Ballow et al. 1996
Immunoglobulin production	Smith and Hayes 1987, Pasatiempo et al. 1989, Semba et al. 1992, Wiedermann et al. 1993b, Semba et al. 1994b
ICAM-1 expression	Bouillon et al. 1991, Wang et al. 1992, Darley et al. 1993, Aoudjit et al. 1994, Aoudjit et al. 1995, Bassi et al. 1995
TNF-α production	Turpin et al. 1990
TGF-β production	Fisher et al. 1992, Szabo et al. 1994
Phospholipase A_2 production	Hope et al. 1990
IFN-γ production	Blalock and Gifford 1976, Blalock and Gifford 1977, Felli et al. 1991, Carman et al. 1992, Wiedermann et al. 1993b, Cantorna et al. 1994, Ikeda et al. 1994, Cantorna et al. 1995, Cantorna et al. 1996
IL-1 production	Trechsel et al. 1985, Dillehay et al. 1988, Gruaz et al. 1990, Matikainen et al. 1991, Tokura et al. 1992
IL-2 production	Colizzi and Malkovský 1985
IL-3 production	Trechsel et al. 1985
IL-4 production	Carman et al. 1992, Cantorna et al. 1994, Cantorna et al. 1995

deficiency have an increased risk of subsequently developing respiratory disease (Sommer et al. 1984).

Vitamin A regulates the terminal differentiation of human keratinocytes (Fuchs and Green 1981) and promotes the synthesis of some keratins while suppressing the synthesis of others (Eckert and Green 1984). In vitamin A–deficient rats, immunohistochemical alterations in cytokeratin expression were observed in the bladder, ureter, kidney, salivary glands, uterus, and conjunctiva before histologic changes could be observed (Gijbels et al. 1992). The expression of keratins is regulated by RAR and thyroid hormone receptors (Tomić-Canić et al. 1996). Mucin gene expression appears to be regulated by all-*trans* retinoic acid (Manna et al. 1994, Manna et al. 1995), although regulation of the different mucin genes by retinoid receptors has not yet been elucidated. Thus, vitamin A, through its metabolites, may regulate keratinization and mucin production on the transcriptional level.

Vitamin A deficiency also causes loss of microvilli, goblet cells, and mucin in the small intestine (DeLuca et al. 1969, Rojanapo et al. 1980). Mucins are large glycoconjugates found on cell surfaces and which are secreted into the lumens of the gastrointestinal, respiratory, and genitourinary tracts. Gastrointestinal mucin is a first line of defense for cells lining the gut. The vitamin A–related pathologic alterations in the gastrointestinal tract may contribute to the increased severity of diarrheal disease in vitamin A–deficient children (Sommer et al. 1984, Barreto et al. 1994).

The genitourinary tract is also affected by vitamin A deficiency. Extensive replacement of normal transitional epithelium with stratified squamous epithelium and the accompanying appearance of distinct types of keratins in the urinary tract have been described in vitamin A–deficient mice (Molloy and Laskin 1988). These mucosal changes might predispose children with vitamin A deficiency to increased urinary tract infections as previously observed (Brown et al. 1979). Vitamin A deficiency also results in altered expression of keratins and metaplastic changes in the endocervix (Darwiche et al. 1993). Whether vitamin A–related alterations in the genital tract increases the risk of sexually transmitted diseases, such as human immunodeficiency virus (HIV) infection, is unknown.

Skin

Skin barrier function is provided by keratinocytes in the epidermis. Vitamin A deficiency induces changes in epidermal keratins (Molloy and Laskin 1985) of which detailed clinical descriptions have been made (Frazier and Hu 1936). Increased skin infections have been described in children with vitamin A deficiency (Spence 1931). An increased susceptibility to skin infections could be caused by changes in keratinization, antigen presentation or other immunologic alterations in skin that may occur during vitamin A deficiency. The relationship between vitamin A status and the pathogenesis of skin infections in humans remains a major gap in current knowledge.

Hematopoiesis

Vitamin A and related retinoids play a central role in the development and differentiation of neutrophils, monocytes, erythrocytes, eosinophils, basophils, megakaryocytes, and lymphocytes (Göttgens and Green 1995) and can modulate T lymphocyte subpopulations in lymphoid tissues and peripheral blood (Semba et al. 1993a). Decreased numbers of circulating lymphocytes and anemia are common during vitamin A deficiency, and vitamin A deficiency is associated with decreased circulating $CD4^+$ lymphocytes in children (Semba et al. 1993a) and adults (Semba et al. 1993b). Supplementation with vitamin A has been shown to increase circulating $CD4^+$ lymphocytes in vitamin A–deficient children (Semba et al. 1993a) and in children with acquired immunodeficiency syndrome (AIDS) (Hussey et al. 1996). The underlying mechanisms by which retinoids influence hematopoietic stem cells and myeloid precursors appears to involve regulation by retinoid receptors (Göttgens and Green 1995).

Apoptosis

Retinoids are potent regulators of cell growth, differentiation, and apoptosis (programmed cell death). Regulation of apoptosis by retinoids may have implications for immune effector cells, virus-infected cells, and tumor cells. All-*trans* retinoic acid and 9-*cis* retinoic acid inhibit activation-driven T lymphocyte and thymocyte apoptosis, suggesting that RXRs may play a role in regulating the negative selection of T lymphocytes in the thymus (Iwata et al. 1992, Yang et al. 1993). In P19 embryonal carcinomal cells, all-*trans* retinoic acid was effective in inducing apoptosis (Horn et al. 1996). Synthetic retinoids selective for RAR induced apoptosis in P19 cells, whereas synthetic retinoids selective for RXR had no effect, and a combination of RAR and RXR ligands resulted in a synergistic increase in apoptosis. This study suggests that both RAR and RXR play a role in apoptosis. Induction of apoptosis in HL-60 cells, a human myeloid leukemia cell line, requires ligand activation of RXR (Nagy et al. 1995). HIV-infected adults who were given daily oral retinoic acid had reduced apoptosis of T lymphocytes, suggesting that retinoid therapy could possibly reduce the decline of $CD4^+$ lymphocytes during HIV infection (Yang et al. 1995). Although these studies suggest a strong role for retinoids in apoptosis, the potential effects of retinoids on apoptosis of immune effector cells still needs further elucidation.

Neutrophils

Neutrophils plays an important role as a first line of defense against infections because they phagocytize and kill bacteria, viruses, parasites, virus-infected cells, and

tumor cells (Twining et al. 1997). However, a reduction in circulating neutrophils, or neutropenia, does not appear to be a feature of clinical vitamin A deficiency. During vitamin A deficiency, neutrophils appear to have impaired function, which could result in initial exposure to a greater number of pathogenic organisms. In vitamin A–deficient rats, the total number of circulating neutrophils was normal; however, a higher proportion of neutrophils were hypersegmented. Functional studies showed defects in chemotaxis, adhesion, phagocytosis, and ability to generate active oxidant molecules in neutrophils from vitamin A–deficient animals compared with controls (Twining et al. 1997). A decrease in neutrophil cathepsin G, which is involved in degradation of phagocytosed material, was found in neutrophils from vitamin A–deficient animals (Twining et al. 1996b).

Natural Killer Cells

Natural killer (NK, or CD16/CD56) cells are found in blood, liver, and spleen and are responsible for "natural cytotoxicity," i.e., antiviral and antitumor immunity that is not MHC-restricted. Vitamin A deficiency reduces the numbers of NK cells and impairs their cytolytic activity (Zhao et al. 1994a, Zhao and Ross 1995). In rats, retinoic acid repletion increased NK cells in peripheral circulation and also increased NK cell cytotoxicity (Zhao and Ross 1995). A recent clinical trial showed that high-dose vitamin A supplementation was associated with a large increase in circulating NK cells in children with AIDS (Hussey et al. 1996). A synthetic retinoid, N-(4-hydroxyphenyl)-retinamide, increased cytolytic activity of NK cells in rats (Zhao et al. 1994b).

Monocytes/Macrophages

Cells of the monocyte/macrophage lineage play a central role in immunity as antigen-presenting cells and as generators of oxygen and nitrogen radicals (Guzman et al. 1991). Monocytes circulate in the blood and then are distributed to different tissues where they contribute to the macrophage population. Macrophages rapidly take up bacteria and viruses, process them, and present antigen to $CD4^+$ lymphocytes in an MHC II-restricted manner. Vitamin A and related retinoids influence monocyte differentiation and function; most of these effects have been studied in leukemic myelomonocytic cell lines such as HL-60, U-937, and THP-1 (Breitman et al. 1989, Öberg et al. 1993, Hemmi and Breitman 1985). In the differentiation of U-937 cells, retinoic acid increased the expression of CD23, a receptor for IgE that plays a role in the regulation of B lymphocyte growth, and decreased the expression of CD14, a receptor for monocyte adhesion during inflammation (Öberg et al. 1993). Vitamin D_3 had the inverse effect, suggesting that there is a functional antagonism between vitamin D_3 and retinoic acid in the regulation of immune responses by monocytes. Tumor necrosis factor-α (TNF-α), a major inflammatory mediator, is produced in high amounts by activated monocytes/macrophages and has a wide range of effects, including induction of B and T lymphocyte proliferation and stimulation of cell adhesion molecules on endothelial cells. The in vitro release of TNF-α appears to be dependent on all-*trans* retinoic acid (Turpin et al. 1990).

Retinoids have been shown to influence both the number and in vitro activity of macrophages (Katz et al. 1987a). An approximate two-fold increase in phagocytosis was caused by all-*trans* retinoic acid in murine macrophages (Dillehay et al. 1988). All-*trans* retinoic acid increased the production of transforming growth factor-β (TGF-β) by human peripheral blood monocytes and human myelomonocytic cells (Szabo et al. 1994). TGF-β has many effects but is primarily involved in the promotion of wound healing. Increased TGF-β immunoreactivity has also been described in human skin biopsies from individuals using topical retinoic acid (Fisher et al. 1992). Anti-inflammatory activity of retinoids are also suggested by in vitro studies which showed that various retinoids could inhibit phospholipase A_2 and arachidonic acid release from rat peritoneal macrophages (Hope et al. 1990).

All-*trans* retinoic acid has been shown to increase interleukin 1 (IL-1) production by human peripheral blood mononuclear cells, murine macrophage cell lines, and murine macrophages (Trechsel et al. 1985, Dillehay et al. 1988). In human monocytes and in a human myeloid leukemia cell line, all-*trans* retinoic acid appeared to enhance IL-1β expression at the transcriptional level (Matikainen et al. 1991). IL-1 is an important mediator of inflammation that has many effects similar to TNF-α, including stimulation of proliferation by T and B lymphocytes. IL-1 is also produced by keratinocytes and may be modulated by all-*trans* retinoic acid and 13-*cis* retinoic acid (Tokura et al. 1992, Gruaz et al. 1990). The potential influence of retinoids on expression of other cytokines by macrophage/monocytes such as IL-10, IL-12, macrophage inflammatory protein (MIP-1), and MIP-2 is unknown.

Langerhans Cells

Langerhans cells are an important component of the immune defense in the skin, where they function as antigen-presenting cells. Dietary vitamin A increases contact sensitivity to a variety of chemical agents in the murine model, probably due to the increased numbers or function of Langerhans cells (Maisey and Miller 1986, Katz et al. 1987b, Sailstad et al. 1995). In vivo retinoic acid treatment increases the ability of human Langerhans cells to present alloantigen to T lymphocytes and is associated with increases in surface expression of HLA-DR and CD11c, two molecules involved in antigen presentation (Meunier et al. 1994). Topical retinoic acid appears to upregulate cuta-

75

neous antigen-presenting function without inducing autoimmunity. This may have therapeutic implications for improving cutaneous immune responsiveness to vaccines, tumor antigens, and skin infections.

T Lymphocytes

T lymphocytes are major regulatory cells of the immune system and include T helper ($CD4^+$) and T cytotoxic ($CD8^+$) lymphocytes. $CD4^+$ lymphocytes recognize antigen in the presence of MHC class II complexes, and $CD8^+$ lymphocytes recognize cells bearing MHC class I complexes. Human lymphocytes contain both retinol and retinoic acid (Sklan et al. 1995). Activation of T cells appears to require retinol (Garbe et al. 1992). In murine T lymphocytes, all-*trans* retinoic acid stimulated expression of RAR-α and increased antigen-specific T lymphocyte proliferation (Friedman et al. 1993). In human T lymphoblasts, retinoic acid increased expression of IL-2 receptors (Sidell et al. 1993). The role of $CD4^+$ lymphocytes in helping B cells develop into antibody-producing cells is discussed below.

$CD8^+$ lymphocytes are involved in the lysis of cells, such as virus-infected cells, expressing antigens in the presence of MHC class I molecules. Vitamin A deficiency was shown to impair cytotoxic T lymphocyte activity in chickens infected with Newcastle disease virus (Sijtsma et al. 1990). Both $CD4^+$ and $CD8^+$ lymphocytes are involved in allograft rejection, and a standard murine diet supplemented with retinyl acetate (0.5 g/kg) has been shown to increase allograft reaction in mice (Malkovský et al. 1983a). Cytotoxic lymphocyte function during vitamin A deficiency in humans has not been studied. A recent clinical trial showed that oral vitamin A supplementation could increase delayed-type hypersensitivity reactions in infants whose serum vitamin A levels increased to greater than 0.70 μmol/L after supplementation (Rahman et al. 1997). This observation may reflect, in part, vitamin A-related upregulation of lymphocyte and/or Langerhans cell function.

Retinoids are involved in the expression of glycoproteins, including some adhesion molecules involved in lymphocyte function. ICAM-1 (CD54) is cell surface glycoprotein and a member of the immunoglobulin gene superfamily. It is expressed on a wide variety of cells and serves as a ligand for the β-2 integrin molecules leukocyte-function associated antigen-1, LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18). The adhesion system plays a key role in lymphocyte circulation, T lymphocyte cytotoxicity, antigen presentation, leukocyte adhesion to endothelial cells, fibroblasts, and hematopoietic cells. The ICAM-1 gene appears to contain a RARE, which is regulated by retinoic acid through RAR-β and RXR-α (Aoudjit et al. 1994, Aoudjit et al. 1995). Retinoic acid can increase expression of ICAM-1 on various tumor cell lines (Bassi et al. 1995; Wang et al. 1992, Bouillon et al. 1991, Darley et al. 1993), suggesting that retinoic acid could mediate the interaction of the immune system, such as cytotoxic T lymphocytes and NK cells, with recognition of some types of cancer cells.

B Lymphocytes and Antibody Responses

B lymphocytes are involved in the production of immunoglobulin, and the growth and activation of B lymphocytes requires retinol (Buck et al. 1990, Blomhoff et al. 1992). B lymphocytes may also use a metabolite of retinol, 14-hydroxy-4,14-*retro*-retinol, instead of retinoic acid, as a mediator for growth (Buck et al. 1991). The effects of retinol and all-*trans* retinoic acid on immunoglobulin synthesis by B lymphocytes has been studied in human cord blood and adult peripheral mononuclear cells (Israel et al. 1991, Wang et al. 1993a, Wang and Ballow 1993b, Ballow et al. 1996). A T cell-dependent antigen, formalinized Cowan I strain *Staphylococcus aureus*, was used in a model system to induce differentiation of B lymphocytes into immunoglobulin-secreting cells. The addition of all-*trans* retinoic acid augmented the production of immunoglobulin M (IgM) synthesis by cord blood mononuclear cells and the production of immunoglobulin G (IgG) by adult peripheral blood mononuclear cells. Highly purified T lymphocytes incubated with retinoic acid enhanced IgM synthesis by cord blood B lymphocytes, providing evidence that retinoic acid modulates T cell help through cytokine production (Ballow et al. 1996). In the murine model, all-*trans* retinoic acid was more active than retinyl acetate, retinaldehyde, or retinol in restoring IgG responses (Chun et al. 1992).

Antibody molecules, or immunoglobulins, play a central role in immune function by attaching to pathogens and recruiting immune effector cells to destroy the pathogens. A hallmark of vitamin A deficiency is the impaired ability to mount an antibody response against T cell-dependent antigens (Smith and Hayes 1987, Semba et al. 1992, Semba et al. 1994b, Wiedermann et al. 1993b) and T cell-independent type 2 antigens, such as pneumoccocal polysaccharide (Pasatiempo et al. 1989). Many of the earlier studies have been summarized elsewhere (Nauss 1996, Ross 1992, Semba 1994a).

Vitamin A deficiency has been hypothesized to influence the balance between T-helper type 2–like and T-helper type 1–like immune responses. *Trichinella spiralis* infection in mice normally stimulates a strong T helper type 2–like response with high levels of parasite-specific IgG and a cytokine profile characterized by IL-4, IL-5, and IL-10 production. In contrast, vitamin A–deficient mice infected with *T. spiralis* produced T helper type 1–like responses that were characterized by low production of parasite-specific IgG and a cytokine profile characterized by interferon-γ (IFN-γ) and IL-12 production(Carman et al. 1992,

76

Cantorna et al. 1994, Cantorna et al. 1995). In vitamin A–deficient rats immunized with β-lactoglobulin, serum IgG, IgM, and IgE, and bile IgA responses against β-lactoglobulin were impaired as compared with pair-fed control animals (Wiedermann et al. 1993b). In vitro lymphocyte stimulation to concanavalin A or ß-lactoglobulin was higher in vitamin A–deficient rats, with higher production of IL-2 and IFN-γ in lymphocyte supernatants, providing additional evidence that vitamin A deficiency increased T-helper type 1–like responses (Wiedermann et al. 1993b). *Hymenolepis nana*, the dwarf tapeworm, is known to elicit a strong T-helper type 1–like response in normal mice. In vitamin A–deficient mice infected with *H. nana*, mesenteric lymph node cells in the presence of soluble egg antigen secreted more IFN-γ but less IL-2 than the same cells from normal mice (Ikeda et al. 1994).

All-*trans* retinoic acid has been shown to decrease IFN production by murine L-929 cells infected with Newcastle disease virus (Blalock and Gifford 1976, Blalock and Gifford 1977). In cloned Th1 cells, physiologic concentrations of all-*trans* retinoic acid downregulated IFN-γ synthesis through the CD28 costimulatory pathway (Cantorna et al. 1996). Downregulation of IL-2 by all-*trans* retinoic acid in a regulatory pathway involving RAR has also been shown in Jurkat T cells (Felli et al. 1991). Modulation of the balance between T-helper type 1 and type 2 responses by retinoids may be influenced by the type of infection. In mice infected with *Mycobacterium bovis*, a standard murine diet supplemented with retinyl acetate, 0.5 g/kg, increased IL-2 production by PPD-stimulated spleen cells and augmented delayed type hypersensitivity (Colizzi and Malkovsky 1985). Pathologic studies revealed that there was a more vigorous inflammatory response in the footpads in vitamin A–supplemented mice 14 days after inoculation with *M. bovis* antigens in the footpad.

Other studies in rats support the idea that certain cytokine signals for B cell activation, proliferation, and production of immunoglobulin are missing during vitamin A deficiency. Vitamin A–deficient rats usually have impaired IgG responses to tetanus toxoid and pneumococcal polysaccharide, however, when these respective antigens were given concomitantly with bacterial lipopolysaccharide, a potent adjuvant (Arora and Ross 1994, Pasatiempo et al. 1994), vitamin A–deficient rats were able to produce a strong antibody response.

Mice with severe combined immunodeficiency (SCID) have been used to study the effects of vitamin A on human antibody responses to tetanus toxoid (Molrine et al. 1995). Peripheral blood lymphocytes from humans previously immunized with tetanus toxoid were used to reconstitute vitamin A–deficient and nondeficient SCID mice. After challenge with tetanus toxoid, vitamin A–deficient SCID mice had a 2.9-fold increase in human anti-tetanus toxoid antibody compared with a 74-fold increase in SCID mice with normal vitamin A status. These data are consistent with the observation that vitamin A supplementation enhances secondary antibody responses to tetanus toxoid in preschool children (Semba et al. 1992).

Cancer Immunity

Vitamin A deficiency has been associated with a higher incidence of cancer and increased susceptibility to carcinogens (Lotan 1996). Vitamin A and related retinoids may increase immunity to tumors by several mechanisms, including enhancement of T cytotoxic lymphocyte activity, NK cell activity, macrophage activity, and apoptosis (Tachibana et al. 1984, Malkovsky et al. 1983b). Alveolar macrophages isolated from vitamin A–supplemented rats had enhanced phagocytosis and tumoricidal activity compared with alveolar macrophages from control rats (Tachibana et al. 1984). Upregulation of HLA-DR expression by all-*trans* retinoic acid has also been described in human breast cancer cells, which also suggests the potential of retinoic acid to induce tumor immunity (Gardner and Walker 1993). All-*trans* retinoic acid has been shown to be beneficial as therapy for acute promyelocytic leukemia (Warrell et al. 1991).

Immunity to Infections

Vitamin A deficiency in children is associated with increased morbidity and mortality (Sommer and West 1986), and these epidemiologic observations are consistent with a large and comprehensive literature describing increased susceptibility to experimental infection in vitamin A–deficient animals (Nauss 1986, Ross 1992, Semba 1994a). As mentioned previously, vitamin A supplementation is widely carried out in many developing countries to improve child survival (Sommer and West 1986). High-dose vitamin A supplementation reduces morbidity and mortality in acute measles (Hussey and Klein 1990) and has become recommended therapy for measles in both developing countries (World Health Organization 1987) and in the United States (Committee on Infectious Diseases 1993).

The encouraging results from community-based clinical trials and hospital-based trials of measles have led to investigations regarding the use of vitamin A supplementation for specific infections. Given the wide range of immune responses to different types of pathogens, it is doubtful that vitamin A will show benefit as disease-targeted therapy for all types of infections. Clinical trials of vitamin A supplementation for respiratory syncytial virus infection have had equivocal results (Bresee et al. 1996, Dowell et al. 1996). Meta-analyses from the recent series of large clinical trials show that vitamin A supplementation reduces the severity of diarrheal disease but has little impact on childhood pneumonia (The Vitamin A and Pneumonia Working Group 1995). High-dose vitamin A supplementation targeted specifically to children with acute diarrhea in

India reduced persistent diarrhea and also reduced the severity of diarrhea among nonbreastfed children (Bhandari et al. 1997). Although vitamin A supplementation was incidentally shown to have little impact on malaria morbidity in Ghana (Binka et al. 1995), clinical trials that are specifically designed to address this issue are needed (Shankar 1996). A recent clinical trial from Papua New Guinea shows that periodic high-dose vitamin A supplementation in preschool children reduces malarial morbidity by one third (Shankar et al. 1997), and a large clinical trial shows that vitamin A supplementation reduces malarial morbidity in pregnant women in Nepal by about 30% (West et al., unpublished data).

Another area in which vitamin A or related retinoids may have therapeutic potential are as therapy for HIV infection. Vitamin A deficiency has been linked with increased mortality (Semba et al. 1993b), higher mother-to-child transmission of HIV (Semba et al. 1994c, Greenberg et al. 1997), increased infant mortality (Semba et al. 1995), child growth failure (Semba et al. 1997a), and higher HIV load in breastmilk (Nduati et al. 1995) and the vagina (Johns et al. 1997, Mostad et al. 1997). Four clinical trials involving over 3000 HIV-infected pregnant women are currently in progress to determine whether antenatal vitamin A supplementation and other micronutrients can reduce mother-to-child transmission of HIV and improve other health outcomes in mothers and infants (Semba 1997b). It is unclear whether vitamin A supplementation will have any value as disease-targeted therapy for HIV infection.

Conclusions

In the last decade, major progress has been made in elucidating the role of vitamin A and related retinoids in immune function. Vitamin A acts via all-*trans* or 9-*cis* retinoic acid or other metabolites and nuclear retinoic acid receptors (RAR, RXR) to regulate gene transcription or may utilize a pathway involving 14-hydroxy-4,14-*retro* retinol. Retinoids influence many aspects of immunity, including mucin and keratin expression, hematopoiesis, apoptosis, the growth, differentiation and function of neutrophils, natural killer cells, monocytes/macrophages, Langerhans cells, T and B lymphocytes, balance between T helper type 1–like and T helper type 2–like immune responses, immunoglobulin production, and expression of cytokines (TGF-β, TNF-α, IFN-γ, IL-1, IL-2, IL-3, IL-4, IL-6, IL-10) and adhesion molecules such as ICAM-1. The results of many of the studies using vitamin A and related retinoids in vitro depend upon cell line, culture conditions, stage of cell differentiation, retinoid concentration, and mode of delivery of the retinoids. Extrapolation to in vivo conditions must be made with caution. However, these observations provide important data to guide future experimental and clinical investigations. The impact of vitamin A deficiency on immunity is more well-established in some immune compartments, including impairment of mucosal immunity by alterations in keratins and mucins, compromised function of accessory cells such as neutrophils, macrophages, and NK cells, alterations in cytokine networks which influence immune responses, and altered antibody responses to T-cell dependent and T-cell independent type 2 antigens. Although great progress has been made in the last decade, regulation by vitamin A and related metabolites on the molecular level of hematopoiesis, apoptosis, generation of specific reactive oxygen species, antigen processing and presentation, CD8 lymphocyte function, cytokine expression, and other aspects of immune function await further clarification.

Acknowledgments. Supported in part by grants from the National Institutes of Health (HD30042, HD32247), the Fogarty International Center, the Thrasher Research Fund, the World Health Organization Expanded Programme on Immunisation, and the United States Agency for International Development (Cooperative Agreement DAN-0045-A-5094-00). The author wishes to thank Anu Shankar and Alan Scott for helpful comments on the manuscript.

Aoudjit F, Bossé J M, Stratowa C, et al (1994) Regulation of intercellular adhesion molecule-1 expression by retinoic acid: analysis of the 5' regulatory region of the gene. Int J Cancer 58:543–9

Aoudjit F, Brochu N, Morin N, et al (1995) Heterodimeric retinoic acid receptor-β and retinoid X receptor-α complexes stimulate expression of the intercellular adhesion molecule-1 gene. Cell Growth Diff 6:515–21

Arora D, Ross AC (1994) Antibody response against tetanus toxoid is enhanced by lipopolysaccharide or tumor necrosis factor-alpha in vitamin A-sufficient and deficient rats. Am J Clin Nutr 59:922–8

Ballow M, Wang W, Xiang S (1996) Modulation of B-cell immunoglobulin synthesis by retinoic acid. Clin Immunol Immunopath 80:S73–81

Barreto ML, Santos LMP, Assis AMO, et al (1994) Effect of vitamin A supplementation on diarrhoea and acute lower-respiratory-tract infections in young children in Brazil. Lancet 344:228–31

Bassi V, Vitale M, Feliciello A, et al (1995) Retinoic acid induces intercellular adhesion molecule-1 hyperexpression in human thyroid carcinoma cell lines. J Clin Endocrinol Metab 80:1129–35

Beaton GH, Martorell R, Aronson K, et al (1993) Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. State-of-the-Art Nutrition Policy Discussion Paper No 13. Geneva: ACC/SCN

Bhandari N, Bahl R, Sazawal S, et al (1997) Breast-feeding status alters the effect of vitamin A treatment during acute diarrhea in children. J Nutr 127:59–63

Binka FN, Ross DA, Morris SS, et al (1995) Vitamin A supplementation and childhood malaria in northern Ghana. Am J Clin Nutr 61:853–9

Blackfan KD, Wolbach SB (1933) Vitamin A deficiency in infants: a clinical and pathological study. J Pediatr 3:679–706

Blalock JE, Gifford G (1976) Suppression of interferon production by vitamin A. J Gen Virol 32:143–7
Blalock JE, Gifford GE (1977) Retinoic acid (vitamin A acid) induced transcriptional control of interferon production. Proc Natl Acad Sci USA 74:5382–6
Blomhoff HK, Smeland EB, Erikstein B, et al (1992) Vitamin A is a key regulator for cell growth, cytokine production, and differentiation in normal B cells. J Biol Chem 267:23988–92
Blumberg B, Bolado J Jr, Derguini F, et al (1996) Novel retinoic acid receptor ligands in *Xenopus* embryos. Proc Natl Acad Sci USA 93:4873–8
Boullon M, Tessier P, Boullanne R, et al (1991) Regulation by retinoic acid of ICAM-1 expression on human tumor cell lines. Biochim Biophys Acta 1097:95–102
Breitman TR, Selonick SE, Collins SJ (1980) Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. Proc Natl Acad Sci USA 77:2936–40
Bresee JS, Fischer M, Dowell SF, et al (1996) Vitamin A therapy for children with respiratory syncytial virus infection: a multicenter trial in the United States. Pediatr Infect Dis J 15:777–82
Brown KH, Gaffar A, Alamgir SM (1979) Xerophthalmia, protein-calorie malnutrition, and infections in children. J Pediatr 95:651–6
Buck J, Ritter G, Dannecker L, et al (1990) Retinol is essential for growth of activated human B cells. J Exp Med 171:1613–24
Buck J, Derguini F, Levi E, et al (1991) Intracellular signaling by 14-hydroxy-4,14-*retro* retinol. Science 254:1654–5
Buck J, Grün F, Kimura S, et al (1993) Anhydroretinol: a naturally occurring inhibitor of lymphocyte physiology. J Exp Med 1993;178:675
Cantorna MT, Nashold FE, Hayes CE (1994) In vitamin A deficiency multiple mechanisms establish a regulatory T helper cell imbalance with excess Th1 and insufficient Th2 function. J Immunol 152:1515–22
Cantorna MT, Nashold FE, Hayes CE (1995) Vitamin A deficiency results in a priming environment conducive for Th1 cell development. Eur J Immunol 25:1673–9
Cantorna MT, Nashold FE, Chun TY, et al (1996) Vitamin A down-regulation of IFN-γ synthesis in cloned mouse Th1 lymphocytes depends on the CD28 costimulatory pathway. J Immunol 1996;156:2674–9
Carlberg C, van Huijsduijnen RH, Staple JK, et al (1994) RZRs, a new family of retinoid-related orphan receptors that function as both monomers and homodimers. Mol Endocrinol 8:757–70
Carman JA, Pond L, Nashold F, et al (1992) Immunity to *Trichinella spiralis* infection vitamin A–deficient mice. J Exp Med 175:111–20
Chambon P (1996) A decade of molecular biology of retinoic acid receptors. FASEB J 10:940–54
Chun TY, Carman JA, Hayes CE (1992) Retinoid repletion of vitamin A–deficient mice restores IgG responses. J Nutr 122:1062–9
Chytil F, Haq RU (1990) Vitamin A mediated gene expression. Crit Rev Eukary Gene Exp 1:61–73
Colizzi V, Malkovský M (1985) Augmentation of interleukin-2 production and delayed hypersensitivity in mice infected with *Mycobacterium bovis* and fed a diet supplemented with vitamin A acetate. Infect Immun 48:581–3
Committee on Infectious Diseases (1993) Vitamin A treatment of measles. Pediatrics 91:1014–5
Darley R, Morris A, Passas J, et al (1993) Interactions between interferon γ and retinoic acid with transforming growth factor β in the induction of immune recognition molecules. Cancer Immunol Immunother 37:112–8
Darwiche N, Celli G, Sly L, et al (1993) Retinoid status controls the appearance of reserve cells and keratin expression in mouse cervical epithelium. Cancer Res 53:2287–99
De Luca L, Little EP, Wolf G (1969) Vitamin A and protein synthesis by rat intestinal mucosa. J Biol Chem 244:701–8
Dillehay DL, Walia AS, Lamon EW (1988) Effects of retinoids on macrophage function and IL–1 activity. J Leuk Biol 44:353–60
Dowell SF, Papic Z, Bresee JS, et al (1996) Treatment of respiratory syncytial virus infection with vitamin A: a randomized, placebo-controlled trial in Santiago, Chile. Pediatr Infect Dis J 15:782–6
Eckert RL, Green H (1984) Cloning of cDNAs specifying vitamin A-responsive human keratins. Proc Natl Acad Sci USA 81:4321–5
Eppinger TM, Buck J, Hämmerling U (1993) Growth control or terminal differentiation: endogenous production and differential activities of vitamin A metabolites in HL-60 cells. J Exp Med 178:1995–2005
Felli MP, Vacca A, Meco D, et al (1991) Retinoic acid-induced down-regulation of the interleukin-2 promotor via *cis*-regulatory sequences containing an octamer motif. Mol Cell Biol 11:4771–8
Fisher GJ, Tavakkol A, Griffiths CEM, et al (1992) Differential modulation of transforming growth factor-β1 expression and mucin deposition by retinoic acid and sodium lauryl sulfate in human skin. J Invest Dermatol 98:102–8
Frazier CN, Hu CK (1936) Nature and distribution according to age of cutaneous manifestations of vitamin A deficiency: a study of two hundred and seven cases. Arch Derm Syph 33:825–52
Friedman A, Halevy O, Schrift M, et al (1993) Retinoic acid promotes proliferation and induces expression of retinoic acid receptor-α gene in murine T lymphocytes. Cell Immunol 152:240–8
Fuchs E, Green H (1981) Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. Cell 25:617–25
Gangopadhyay NN, Moldoveanu Z, Stephensen CB (1996) Vitamin A deficiency has different effects on immunoglobulin A production and transport during influenza A infection in BALB/c mice. J Nutr 126:2960–7
Garbe A, Buck J, Hämmerling U (1992) Retinoids are important cofactors in T cell activation. J Exp Med 176:109–17
Gardner FJE, Walker RA (1993) Modulation of cell surface components of human breast cancer cells by retinoic acid: enhanced HLA-DR expression. J Pathol 170:23–9
Gijbels MJJ, van der Ham F, van Bennekum AM, et al (1992) Alterations in cytokeratin expression precede histological changes in epithelia of vitamin A–defi-

79

cient rats. Cell Tissue Res 268:197–203
Göttgens B, Green AR (1995) Retinoic acid and the differentiation of lymphohaemapoietic stem cells. BioEssays 17:187–9
Green HN, Mellanby E (1928) Vitamin A as an anti-infective agent. BMJ 2:691–6
Greenberg BL, Semba RD, Vink PE, et al (1997) Vitamin A deficiency and maternal-infant transmission of HIV in two metropolitan areas in the United States. AIDS 11:325–32
Gruaz DC, Didierjean L, Gumowski-Sunek D, et al (1990) Effect of topical retinoic acid on the interleukin 1α and β immunoreactive pool in normal human epidermis. Brit J Dermatol 123:283–9
Guzman JJ, Marks JF, Caren LD (1991) In vivo and in vitro studies on the effects of vitamin A on the chemiluminescent response of murine peritoneal exudate cells. Toxicol Lett 57:125–137
Haq RU, Pfahl M, Chytil F (1991) Retinoic acid affects the expression of nuclear retinoic acid receptors in tissues of retinol-deficient rats. Proc Natl Acad Sci 88:8272–6
Hemmi H, Breitman TR (1985) Induction of functional differentiation of a human monocytic leukemia cell line (THP-1) by retinoic acid and cholera toxin. Jpn J Canc Res 76:345–51
Hirose T, Apfel R, Pfahl M, et al (1995) The orphan receptor TAK1 acts as a repressor of RAR-, RXR- and T_3R-mediated signaling pathways. Biochem Biophys Res Comm 211:83–91
Hope WC, Patel BJ, Fiedler-Nagy C, et al (1990) Retinoids inhibit phospholipase A_2 in human synovial fluid and arachidonic acid release from rat peritoneal macrophages. Inflammation 14:543–59
Horn V, Minucci S, Ogryzko VV, et al (1996) RAR and RXR selective ligands cooperatively induce apoptosis and neuronal differentiation in P19 embryonal carcinoma cells. FASEB J 10:1071–7
Hussey GD, Klein M (1990) A randomized, controlled trial of vitamin A in children with severe measles. N Engl J Med 323:160–4
Hussey G, Hughes J, Potgieter S, et al (1996) Vitamin A status and supplementation and its effects on immunity in children with AIDS. Abstracts of the XVII International Vitamin A Consultative Group Meeting, Guatemala City. Washington, DC: International Life Sciences Institute, p 6
Ikeda K, Matsuo S, Asano K, et al (1994) Modulation of cytokine secretion by mesenteric lymph node cells from vitamin A–deficient mice during *Hymenolepis nana* infection. In Vivo 8:1015–8
Israel H, Odziemiec C, Ballow M (1991) The effects of retinoic acid on immunoglobulin synthesis by human cord blood mononuclear cells. Clin Immunol Immunopath 59:417–25
Iwata M, Mukai M, Nakai Y, et al (1992) Retinoic acids inhibit activation-driven apoptosis in T cell hybridomas and thymocytes. J Immunol 149:3302–8
Johns GC, Nduati RW, Mbori-Ngacha D, et al (1997) Genital shedding of human immunodeficiency virus type 1 DNA during pregnancy: association with immunosuppression, abnormal cervical or vaginal discharge, and severe vitamin A deficiency. J Infect Dis 175:57–62
Kastner P, Mark M, Chambon P (1995) Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? Cell 83:859–69
Katz DR, Drzymala M, Turton JA, et al (1987a) Regulation of accessory cell function by retinoids in murine immune responses. Brit J Exp Path 68:343–50
Katz DR, Mukherjee S, Maisey J, et al (1987b) Vitamin A acetate as a regulator of accessory cell function in delayed-type hypersensitivity responses. Int Archs Allergy Appl Immun 82:53–6
Lotan R (1996) Retinoids in cancer chemoprevention. FASEB J 10:1031–9
Maisey J, Miller K (1986) Assessment of the ability of mice fed on vitamin A supplemented diet to respond to a variety of potential contact sensitizers. Contact Dermatitis 15:17–23
Malkovský M, Edwards AJ, Hunt R, et al (1983a) T-cell-mediated enhancement of host-versus-graft reactivity in mice fed a diet enriched in vitamin A acetate. Nature 302:338–40
Malkovský M, Doré J C, Hunt R, et al (1983b) Enhancement of specific antitumor immunity in mice fed a diet enriched in vitamin A acetate. Proc Natl Acad Sci USA 80:6322–6
Manna B, Lund M, Ashbaugh P, et al (1994) Effect of retinoic acid on mucin gene expression in rat airways in vitro. Biochem J 297:309–13
Manna B, Ashbaugh P, Bhattacharyya SN (1995) Retinoic-acid regulated cellular differentiation and mucin gene expression in isolated rabbit tracheal epithelial cells in culture. Inflammation 19:489–502
Matikainen S, Serkkola E, Hurme M (1991) Retinoic acid enhances IL-1β expression in myeloid leukemia cells and in human monocytes. J Immunol 147:162–7
McDowell EM, Keenan KP, Huang M (1984) Effects of vitamin A–deprivation on hamster tracheal epithelium: a quantitative morphologic study. Virchows Arch B 45:197–219
Meunier L, Bohjanen K, Voorhees JJ, et al (1994) Retinoic acid upregulates human Langerhans cell antigen presentation and surface expression of HLA-DR and CD11c, a β2 integrin critically involved in T–cell activation. J Invest Dermatol 103:775–9
Molloy CJ, Laskin JD (1985) Alterations in mouse epidermal keratin production induced by dietary vitamin A deficiency. Ann N Y Acad Sci 455:739–40
Molloy CL, Laskin JD (1988) Effect of retinoid deficiency on keratin expression in mouse bladder. Exp Mol Pathol 49:128–40
Moirine DC, Polk DB, Ciamarra A, et al (1995) Impaired human responses to tetanus toxoid in vitamin A–deficient SCID mice reconstituted with human peripheral blood lymphocytes. Infect Immun 63:2867–72
Mostad SB, Overbaugh J, DeVange DM, et al (1997) Hormonal contraception, vitamin A deficiency, and other risk factors of shedding of HIV-1 infected cells from the cervix and vagina. Lancet 350:922–7
Nagy L, Thomázy VA, Shipley GL, et al (1995) Activation of retinoid X receptors induces apoptosis in HL-60 cell lines. Mol Cell Biol 15:3540–51
Napoli JL (1996) Retinoic acid biosynthesis and metabolism. FASEB J 10:993–1001
Nauss KM (1986) Influence of vitamin A status on the immune system. In: Bauernfeind JC (ed), Vitamin A

80

deficiency and its control. Orlando, FL: Academic Press, pp 207–43
Nduati RW, John GC, Richardson BA, et al (1995) Human immunodeficiency virus type 1-infected cells in breast milk: association with immunosuppression and vitamin A deficiency. J Infect Dis 172:1461–8
Öberg F, Botling J, Nilsson K (1993) Functional antagonism between vitamin D_3 and retinoic acid in the regulation of CD14 and CD23 expression during monocytic differentiation of U-937 cells. J Immunol 150:3487–95
Ortiz MA, Piedrafita FJ, Pfahl M, et al (1995) TOR: a new orphan receptor expressed in the thymus that can modulate retinoid and thyroid hormone signals. Mol Endocrinol 9:1679–91
Pasatiempo AMG, Bowman TA, Taylor CE, et al (1989) Vitamin A depletion and repletion: effects on antibody response to the capsular polysaccharide of Streptococcus pneumoniae, type III. Am J Clin Nutr 49:501–10
Pasatiempo AMG, Kinoshita M, Foulke DT, et al (1994) The antibody response of vitamin A–deficient rats to pneumococcal polysaccharide is enhanced through coimmunization with lipopolysaccharide. J Infect Dis 169:441–4
Pfahl M, Chytil F (1996) Regulation of metabolism by retinoic acid and its nuclear receptors. Annu Rev Nutr 16:257–83
Rahman MM, Mahalanabis D, Alvarez JO, et al (1997) Effect of early vitamin A supplementation on cell-mediated immunity in infants younger than 6 mo. Am J Clin Nutr 65:144–8
Retnakaran R, Flock G, Giguère V (1994) Identification of RVR, a novel orphan nuclear receptor that acts as a negative transcriptional regulator. Mol Endocrinol 8:1234–44
Rojanapo W, Lamb AJ, Olson JA (1980) The prevalence, metabolism and migration of goblet cells in rat intestine following the induction of rapid, synchronous vitamin A deficiency. J Nutr 110:178–88
Ross AC (1992) Vitamin A status: relationship to immunity and the antibody response. Proc Soc Exp Biol Med 200:303–20
Sallstad DM, Krishnan SD, Tepper JS, et al (1995) Dietary vitamin A enhances sensitivity of the local lymph node assay. Toxicology 96:157–63
Scrimshaw NS, Taylor CE, Gordon JE (1968) Interactions of nutrition and infection. Geneva: World Health Organization
Semba RD, Muhilal, Scott AL, et al (1992) Depressed immune response to tetanus in children with vitamin A deficiency. J Nutr 122:101–7
Semba RD, Muhilal, Ward BJ, et al (1993a) Abnormal T-cell subset proportions in vitamin A–deficient children. Lancet 341:5–8
Semba RD, Graham NMH, Calaffa WT, et al (1993b) Increased mortality associated with vitamin A deficiency during human immunodeficiency virus type 1 infection. Arch Intern Med 153:2149–54
Semba RD (1994a) Vitamin A, immunity, and infection. Clin Infect Dis 19:489–99
Semba RD, Muhilal, Scott AL, et al (1994b) Effect of vitamin A supplementation on IgG subclass responses to tetanus toxoid in children. Clin Diag Lab Immunol 1:172–5
Semba RD, Miotti PG, Chiphangwi JD, et al (1994c) Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. Lancet 343:1593–7
Semba RD, Miotti PG, Chiphangwi JD, et al (1995) Infant mortality and maternal vitamin A deficiency during HIV infection. Clin Infect Dis 21:966–72
Semba RD, Miotti PG, Chiphangwi JD, et al (1997a) Maternal vitamin A deficiency and child growth failure during human immunodeficiency virus infection. J Acquired Immun Defic Syndr 14:219–22
Semba RD (1997b) Vitamin A and human immunodeficiency virus infection. Proc Nutr Soc 56:1–11
Shankar AH (1996) Vitamin A and malaria [letter]. Am J Clin Nutr 62:842
Shankar AH, Genton B, Semba RD, et al (1997) Vitamin A supplementation as a nutrient-based intervention to reduce malarial morbidity. Abstracts of the XVIII International Vitamin A Consultative Group Meeting, Cairo, Egypt, September 22–26, 1997, p 31
Sidell N, Chang B, Bhatti L (1993) Upregulation by retinoic acid of interleukin-2-receptor mRNA in human T lymphocytes. Cell Immunol 146:28–37
Sijtsma SR, Rombout JHWM, West CE, et al (1990) Vitamin A deficiency impairs cytotoxic T lymphocyte acitivity in Newcastle disease virus-infected chickens. Vet Immunol Immunopath 26:191–201
Sklan D, Trifon S, Kedar O, et al (1995) Retinoid metabolism in human leukocytes. Brit J Nutr 73:889–95
Smith SM, Hayes CE (1987) Contrasting impairments in IgM and IgG responses of vitamin A–deficient mice. Proc Natl Acad Sci USA 84:5878–82
Sommer A, Katz J, Tarwotjo I (1984) Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. Am J Clin Nutr 40:1090–5
Sommer A, West KP Jr (1986) Vitamin A deficiency: health, survival, and vision. New York: Oxford University Press
Spence JC (1931) A clinical study of nutritional xerophthalmia and night-blindness. Arch Dis Child 6:17–26
Stephensen CB, Moldoveanu Z, Gangopadhyay NN (1996) Vitamin A deficiency diminishes the salivary IgA response and enhances the serum IgG response to influenza A virus infection in BALB/c mice. J Nutr 126:94–102
Sweet KL, K'ang HJ (1935) Clinical and anatomic study of avitaminosis A among the Chinese. Am J Dis Child 50:699–734
Szabo S, Puppolo M, Verma B, et al (1994) Regulatory potential of ethanol and retinoic acid on human monocyte functions. Alcohol Clin Exp Res 18:548–54
Tachibana K, Sone S, Tsubura E, et al (1984) Stimulatory effect of vitamin A on tumoricidal activity of rat alveolar macrophages. Brit J Cancer 49:343–8
Tokura Y, Edelson RL, Gasparro FP (1992) Retinoid augmentation of bioactive interleukin-1 production by murine keratinocytes. Brit J Dermatol 126:485–95
Tomić-Canić M, Day D, Samuels HH, et al (1996) Novel regulation of keratin gene expression by thyroid hormone and retinoid receptors. J Biol Chem 271:1416–23
Tran P, Zhang XK, Salbert G, et al (1992) COUP orphan receptors are negative regulators of retinoic acid

81

response pathways. Mol Cell Biol 12:4666–76
Trechsel U, Evêquoz V, Fleisch H (1985) Stimulation of interleukin 1 and 3 production by retinoic acid in vitro. Biochem J 230:339–44
Tseng SCG, Hatchell D, Tierney N, et al (1984) Expression of specific keratin markers by rabbit corneal, conjunctival, and esophageal epithelia during vitamin A deficiency. J Cell Biol 99:2279–86
Turpin J, Mehta K, Blick M, et al (1990) Effect of retinoids on the release and gene expression of tumor necrosis factor-α in human peripheral blood monocytes. J Leuk Biol 48:444–50
Twining SS, Zhou X, Schulte DP, et al (1996a) Effect of vitamin A deficiency on the early response to experimental *Pseudomonas* keratitis. Invest Ophthalmol Vis Sci 37:511–22
Twining SS, Schulte DP, Wilson PM, et al (1996b) Neutrophil cathepsin G is specifically decreased under vitamin A deficiency. Biochim Biophys Acta 1317:112–8
Twining SS, Schulte DP, Wilson PM, et al (1997) Vitamin A deficiency alters rat neutrophil function. J Nutr 127:558–65
Van Agtmaal EJ (1989) Vitamin A and protein in tear fluid: a nutritional field survey on preschool children in northeast Thailand. (Thesis) Amsterdam: University of Amsterdam
The Vitamin A and Pneumonia Working Group (1995) Potential interventions for the prevention of childhood pneumonia in developing countries: a meta-analysis of data from field trials to assess the impact of vitamin A supplementation on pneumonia morbidity and mortality. Bull WHO 73:609–19
Wang Z, Cao Y, D'Urso CM, et al (1992) Differential susceptibility of cultured human melanoma cell lines to enhancement by retinoic acid of intercellular adhesion molecule 1 expression. Cancer Res 52:4766–72
Wang W, Napoli JL, Ballow M (1993a) The effects of retinol on in vitro immunoglobulin synthesis by cord blood and adult peripheral blood mononuclear cells. Clin Exp Immunol 92:164–8
Wang W, Ballow M (1993b) The effects of retinoic acid on in vitro immunoglobulin synthesis by cord blood and adult peripheral blood mononuclear cells. Cell Immunol 148:291–300
Warrell RP Jr, Frankel SR, Miller WH Jr, et al (1991) Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-*trans* retinoic acid). N Engl J Med 324:1385–93
Wiedermann U, Hanson LÅ, Holmgren J, et al (1993a) Impaired mucosal antibody response to cholera toxin in vitamin A–deficient rats immunized with oral cholera vaccine. Infect Immun 61:3952–7
Wiedermann U, Hanson LC, Kahu H, et al (1993b) Aberrant T-cell function in vitro and impaired T-cell dependent antibody response in vivo in vitamin A–deficient rats. Immunology 80:581–6
Wilhelm DL (1954) Regeneration of the tracheal epithelium in the vitamin-A-deficient rat. J Pathol Bact 67:361–5
Wong YC, Buck RC (1971) An electron microscopic study of metaplasia of the rat tracheal epithelium in vitamin A deficiency. Lab Invest 24:55–66
World Bank (1993) World Bank Reports 1993: Investing in health. New York: Oxford University Press
World Health Organization (1987) Joint WHO/UNICEF statement on vitamin A for measles. WHO Wkly Epidemiol Rec 62:133–4
Yang Y, Vacchio MS, Ashwell JD (1993) 9-*cis*-retinoic acid inhibits activation-driven T-cell apoptosis: implications for retinoid X receptor involvement in thymocyte development. Proc Natl Acad Sci USA 90:6170–4
Yang Y, Bailey J, Vacchio MS, et al (1995) Retinoic acid inhibition of *ex vivo* human immunodeficiency virus-associated apoptosis of peripheral blood cells. Proc Natl Acad Sci USA 92:3051–5
Zhao Z, Murasko DM, Ross AC (1994a) The role of vitamin A in natural killer cell cytotoxicity, number and activation in the rat. Nat Immun 13:29–41
Zhao Z, Matsuura T, Popoff K, et al (1994b) Effects of N-(4-hydroxyphenyl)-retinamide on the number and cytotoxicity of natural killer cells in vitamin-A-sufficient and -deficient rats. Nat Immun 13:280–8
Zhao Z, Ross AC (1995) Retinoic acid repletion restores the number of leukocytes and their subsets and stimulates natural cytotoxicity in vitamin A–deficient rats. J Nutr 125:2064–73

82

Eating from a Shared Plate Affects Food Consumption in Vitamin A-Deficient Nepali Children[1,2]

A. V. Shankar,*[3] J. Gittelsohn,* K. P. West, Jr.,* R. Stallings,* T. Gnywali** and F. Faruque†

*Center for Human Nutrition and Division of Human Nutrition; †Department of International Health, School of Public Health, Johns Hopkins University, Baltimore, MD 21205; and **Nepal Nutrition Intervention Project-Sarlahi, Nepal Eye Hospital, Kathmandu, Nepal

ABSTRACT This case-control study evaluates the relationship between shared-plate eating behavior in young Nepali children (aged 1–6 y) and risk of vitamin A deficiency. Day-long observations of dietary practices were conducted on 7 d over a 15-mo period in 162 households: 81 households with a child with a known history of mild xerophthalmia (cases) were matched with 81 having a non-xerophthalmic child of similar age (controls). Shared-plate eating occurred in 25% of all feeding episodes and at least once in 65% of all days observed. Overall, children engaging in shared-plate eating were significantly more likely to consume grains, vegetables, carotenoid-rich vegetables, pulses, fruits, meats and fish, and dairy products and had significantly larger portion sizes for grains, vegetables, fruits, pulses and dairy products, compared with children who ate alone. In general, feeding behaviors between case and control children tended to be similar. However, shared-plate feeding episodes among case children were significantly less likely to include meats or fish [odds ratio (OR) = 0.5, confidence interval (CI) = 0.3–0.8], dairy products (OR = 0.6, CI = 0.4–0.9) or pulses (OR = 0.7, CI = 0.5–1.0). Individual-plate feeding episodes among case children were more likely to include vegetables (OR = 1.3, CI = 1.0–1.6) than those of control children. Case children were more likely to share a plate with a male adult (OR = 1.7, CI = 1.0–2.8), but less likely to eat from a plate shared with females of any age compared with controls (female adult: OR = 0.6, CI = 0.4–0.9; female child: OR = 0.6, CI = 0.4–1.0). Shared-plate eating may benefit a young child's dietary intake, but the identity of the food sharer may modify this influence. J. Nutr. 128: 1127–1133, 1998.

KEY WORDS: • *shared-plate eating* • *vitamin A deficiency* • *children* • *Nepal* • *food sharing*

Food sharing, a nearly universal practice (Goldschmidt 1966), refers to a complex of cultural rules and behaviors governing the distribution and redistribution of food among individuals. A common food-sharing practice may be termed shared-plate eating, wherein foods are presented to two or more individuals on a single bowl or plate and are shared among the group. Although a substantial amount of work has been done on food sharing both within and between households (Blurton Jones 1984, Engle 1990, Feinman 1979, Pelto 1984, Rogers and Schlossman 1990, Van Esterik 1985), limited data are available that examine dietary intake in a shared-plate situation or relate this common practice to nutritional risk.

The feeding of young children differs substantially among cultures (Dettwyler 1989); from studies of intrahousehold food allocation related to food sharing, we know that household behaviors can result in certain individuals receiving disproportionately more or less of the household food supply. Most commonly, females are reported to receive less food of "poorer quality" than their male counterparts (Basu et al. 1986, Carloni 1981, Chen et al. 1981, Gittelsohn 1989 and 1991). In Guatemala, male heads of households received proportionally more dietary protein, whereas female heads of household consumed more energy than other household members (Engle and Nieves 1993). Other researchers have found that although female adults may consume poorer quality foods, their female children were not discriminated against in this manner (Abdullah and Wheeler 1985, Basu et al. 1986, Gittelsohn 1991, Harriss 1990).

The specific cultural rules that govern intrahousehold food allocation patterns can also influence shared-plate eating. For example, the status of the food consumer within the household often determines the social interactions during mealtime (Panter-Brick 1995, Van Esterik 1985). If female children are generally served after older males, based on the social hierarchy of the family, a young girl may be less likely to reach for choice foodstuff (e.g., meat) first if eating with an older brother.

Much of the literature that does exist on shared-plate eating comes from ethnographic case studies. These have shown that general societal norms promoting beneficial exchange and cooperation are often echoed within the etiquette surrounding the sharing of food. For example, in Zimbabwe, children learn

[1] Funded through Cooperative Agreement No. DAN 0045-A-00-5094-00 between the Center for Human Nutrition/Dana Center for Preventive Ophthalmology, The Johns Hopkins University and The Office of Health and Nutrition, U.S. Agency for International Development, with additional support from Task Force Sight and Life (Roche, Basel), and National Institutes of Health shared instrument grant S10-RR 04060.

[2] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[3] To whom correspondence and reprint requests should be addressed.

0022-3166/98 $3.00 © 1998 American Society for Nutritional Sciences.

Manuscript received 19 September 1997. Initial review completed 29 October 1997. Revision accepted 16 February 1998.

83

early who eats first and who eats with whom, based on their social standing (Farb and Armelogos 1980). In The Gambia, food-sharing etiquette requires that those sharing from the same bowl wait for the senior among them to invite them to start the meal and never encroach on their neighbor's portion (Beckerleg 1995). In a study in the hill regions of Western Nepal, Gittelsohn (1989) found that shared-plate eating occurred infrequently, possibly the result of beliefs surrounding the pollution of food that occurs by touching another's served food. In this setting, shared-plate eating also seemed to reflect economic status, with members of poorer households more likely to consume food from the same plate.

Other factors such as the characteristics and dynamics of a shared-plate episode may also directly influence an individual's consumption. Such characteristics include the number of people involved; the age, sex, identity (e.g., mother) and health of each participant; the type and amount of food shared; and the sequence in which the sharing occurs (i.e., did they begin eating together or did one or more come later?). Young children who compete with others in a shared-plate situation may be less able to procure food because of their smaller size. In households in which food is scarce, intense competition may place a child at an even greater disadvantage. On the other hand, other factors can ensure that a child will receive his/her due portion. For example, mothers or others may actively encourage small or sick children to eat more food (Engle and Zeitlin 1996). The effects of food sharing on the nutritional status of children or women, however, remains unknown.

The dearth of published literature on this subject is due in part to the methodological difficulty of examining the dynamics of shared-plate eating. The only way to capture actual behaviors is to observe specific mealtime interactions. One published study in The Gambia (Hudson 1995) estimated food intake (actually energy intake) from shared bowls of food on the basis of a distribution algorithm that used a nonproportional relationship between energy requirements and body weights. The validity of this algorithm was tested against total energy expenditure (as determined by measuring doubly-labeled water) and found to be within 80% of total energy expenditure. Although this may be suitable to estimate total energy intake within a shared-plate setting, it does not assess intakes of micronutrients, such as vitamin A.

In this paper we use highly specific event-sampled data from multiple day-long continuous observations to characterize shared-plate eating in a rural population of Nepali children among whom this practice is common. The nutritional risks of shared-plate eating are examined in children at high risk of vitamin A deficiency (having a known previous history of xerophthalmia) compared with lower risk children having no history of xerophthalmia.

SUBJECTS AND METHODS

This study was conducted in three village development committees (formally panchayats) with a population of ~15,000 in the Sarlahi District, located in the central lowland region of Nepal. In January 1992, we conducted a community-wide ocular survey of 3735 children (aged 1–6 y) from 2389 households. A detailed description of the methodology has been published elsewhere (Shankar 1995). Children with clinical signs of xerophthalmia or a current history of night blindness were identified by senior ophthalmic assistants. Eighty-six households had at least one xerophthalmic child between the ages of 1 and 6 y. Cases were treated with high-potency vitamin A according to the WHO guidelines (Sommer 1982) and referred to the local health post for follow-up. No nutritional education or counseling was provided to the parents or guardians regarding the cause or prevention of vitamin A deficiency at the time of diagnosis. Five families either refused to participate in the study or migrated from the district, resulting in 81 households enrolled in the study. Each clinically diagnosed child between the ages of 1 and 6 y served as the "case focus child" in the household.

Controls were selected from the pool of households whose surveyed children exhibited no xerophthalmia and for which there was no reported history of night blindness in any sibling or the child's mother. Control households were matched to case households based on the age of a control to a case focus child (±3 mo) and on age of the next youngest sibling (±4 mo), if present. Matching by younger sibling was done to adjust a priori for potential confounding influences in the feeding behavior of a focus child that could result from the presence of a younger sibling in the household and to examine feeding behaviors of younger siblings from high risk and lower risk households.

Following the survey and selection, the 162 households were monitored longitudinally for 15 mo using day-long direct continuous observations to assess dietary intake and related behaviors. Study households were visited seven times from March 1993 to June 1994, with each visit ~2 mo apart. Our observers followed the focus children for ~8 h during the day, covering the two major meals and much of the day-long snacking. Of the 162 households enrolled, 144 completed all seven visits, another 10 had six visits and the remaining 8 households had fewer than six. Ten local male Nepalese whom we trained for 3 mo in observational techniques and food weight estimation served as our observers. Observers were masked to their household's pretreatment status.

Each observer was responsible for collecting data for an average of nine case-control household pairs (18 total) over the 7 d of observation. The observers were required to prioritize their recording of behaviors with respect to individuals and activities. Food consumption by the focus child had the highest priority. The observers used event sampling (Bentley et al. 1994) to record over 110 different behaviors of interest. If there was no change in the child's behavior over an extended period of time (e.g., sleeping or playing), the observers recorded the behavior every 5 min regardless. The behavioral codes were broadly categorized into seven groups including hygiene-related (12 codes), food-related (36), child care (14), talking (14), work (14), general activities (12) and observer-related (5). To facilitate memorization by the observers, all codes were 5 letter mnemonic codes created from Nepali descriptions of the behavior of interest.

The dietary data included herein represent food consumed during the observational periods only. In the case of individual-plate eating, observers visually estimated the amount of food served and food lost to spillage or other means. The total food-specific portion size for that feeding episode was calculated based on the estimated amount served minus the estimated food lost. During shared-plate feeding episodes, observers would visually estimate the food-specific totals served on the plate and the total consumed by each person, except for the last person eating on the shared-plate. The portions for the last person in the shared-plate feeding episode were calculated by subtracting the amount consumed by every other person eating from the shared-plate from the total amount of food served on the plate. Each line of dietary data included the food name, unit of measurement (grams), whether it was served, consumed or lost, the identities of the server and recipient of the food, and the food quality or condition (e.g., watery pulses or meat with mostly bones). In the case of food mixtures from a single recipe, only the two most prominent foods were listed, in order of decreasing quantity. In the case of non-recipe food mixtures, such as rice, pulses and vegetables, each food (or two most prominent ingredients of that food) was recorded, in order; the total consumed was specified as a food mix and each food assigned a quantity. Each specific food was then categorized within a food group. The following food groups were used: grains (e.g., rice, corn or wheat); vegetables with low or no carotenoid content (e.g., white gourds, cauliflower or potatoes); carotenoid-rich vegetables (e.g., dark green leafy vegetables or pumpkin); pulses (e.g., lentils or chick peas); fruits with low or no carotenoid content (e.g., apples, pears or bananas); carotenoid-rich fruits (e.g., mango, papaya or jackfruit); meats or fish (e.g., chicken, goat or pond fish); and dairy products (e.g., milk or yogurt). Specific observational training procedures used in the study have been previously described (Gittelsohn et al. 1994).

Assessment of the shared-plate eating event. Shared-plate eating events were recorded in two ways. Once it was clear that two or more

84

individuals were eating from a single plate, a specific activity code was used to signify that the actor and recipient were eating together. This code was assigned to all members of the shared-plate episode and also to individuals who joined a meal in progress. The second method for recording food eaten in a shared-plate setting was to use a special food condition code for every food that was eaten in a shared-plate setting. This double-coding system enabled us to readily identify shared-plate episodes.

A feeding episode was defined to be all foods consumed by the focus child, as part of a meal or a snack, not more than 30 min apart. We created an operational definition of a meal, based on the assumption that, within the Nepali context, meals tend to involve several individuals consuming a number of different foods together. A meal was defined as any feeding episode that involved three or more individuals and involved consumption of five or more distinct foods. On the basis of this definition, 74% of the feeding episodes (n = 4277) observed were classified as meals, whereas the remaining 26% were classified as snacks.

Shared-plate feeding episodes were categorized by age and sex of at least one sharer, excluding the focus child. Because there may be more than one food sharer in any shared-plate feeding episode, these are nonexclusive groups. We also noted the extent of interplate food sharing, which is defined as food already served to an individual in one plate that is given to another recipient having another plate.

Statistical analyses. Observations were quantified at the level of the feeding episode (meals and snacks) for various outcomes including the estimated quantity in grams consumed for each of eight food groups or the presence or absence of each food group. Because each focus child was observed on multiple days and during multiple meals per day, these outcome measures are expected to be positively correlated within children.

To adjust for within-child correlation, we used one of two approaches. First, for binary responses, including the presence or absence of each food group, we computed robust variances based on the generalized estimating equations for marginal models (Zeger et al. 1988), assuming equivalent correlations. Second, for normally distributed response variables, such as the estimated quantities consumed for each food group, we assumed a random coefficients model, allowing a separate slope for each child, a repeated structure for observation days and an autoregressive correlation between meals within a day. Statistical differences were calculated on the basis of the difference between the least-square means. A Scheffé's adjustment was used to account for multiple comparisons. These regressions were run using PROC MIXED (SAS Institute 1995).

Odds ratios (OR) were adjusted for the sex of the focus child and socioeconomic status (SES) of the household, sequence of the observation day and feeding episode within the day. The SES score was based on an additive scale comprised of material possessions and house quality. The final scale included the following eight items (in currently usable condition): flashlights (1+), watch (1+), cycle (1+), armoire (1+), beds (2+), number of rooms in the house (2+), a roof made with more than just straw and irrigation pumpset. The α coefficient for this scale was 0.78, the mean score value was 3. Households were categorized as having relatively higher SES on the basis of an SES scale score >3.

Verbal informed consent from head of households and mothers was obtained before their participation in the survey and again after selection in the case-control study. This study was approved by the Nepal Health Research Council, Kathmandu, Nepal and the Committee on Human Research, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

RESULTS

Classification of child feeding episodes. As a prerequisite to determining differences in feeding patterns between case and control children, it was necessary to develop a generalized classification paradigm for feeding episodes. Table 1 categorizes feeding episodes by type of food sharing, eating location and meal type (snack or meal) for both case and control households. No food sharing occurred in 38% of all feeding episodes (meals) and 22% of all snacks. Shared-plate eating was the predominant type of food sharing (26% of all feeding episodes) compared with interplate food sharing (14%). Shared-plate eating in study households occurred nearly exclusively at meals within the home (24% of all feeding episodes).

TABLE 1

Feeding patterns for all study children includes type of food sharing, whether a meal or snack, and location of feeding episode[1]

Type of food sharing	(% of feeding episodes n = 4277)	Location of feeding episode	Meal or snack	Percentage of total
No food sharing	(59.6)	Within (54.4)	Meal	37.6
			Snack	16.8
		Outside (5.2)	Meal	0.5
			Snack	4.7
Shared-plate eating	(26.3)	Within (25.9)	Meal	23.9
			Snack	2.0
		Outside (0.4)	Meal	0.4
			Snack	0.0
Interplate sharing	(14.1)	Within (13.4)	Meal	11.4
			Snack	2.0
		Outside (0.7)	Meal	0.2
			Snack	0.5

[1] Location is dichotomized as either within or around the focus child's housing compound or outside or at another's household.

The majority of shared-plate feeding episodes (88%) can be described as multiple simultaneous sharing in which all individuals are served at the same time. The food was not divided into sections when initially served; individuals tended to separate, mix and consume their own portion and, in general, had equal access to the food provided. At any time during the meal, any member of the meal group could request more food. Each member leaves the shared plate as they finished. Post-served sharing (7%) occurs when an individual begins eating and another individual sits down later to eat, often separating a portion of the mixed food for him/herself. The remaining 5% of shared plate feeding episodes were classified as multiple post-served sharing. In this case, the newest addition to the shared plate must acquire food from either individuals currently eating off the plate or ask for more food. There were no differences in the frequency of the above-described types of food sharing by case status.

Shared-plate vs. individual plate eating. We first examined the effect of shared-plate eating on the variety and quality of food consumption in both groups of focus children. For these analyses, individual-plate eating includes both feeding episodes in which no food sharing or only interplate food sharing occurred. In general, a child eating from a shared plate was more than two times as likely to consume any of the food groups examined compared with children eating alone (Table 2). Specifically, this included grains, vegetables, carotenoid-rich vegetables, pulses, meats and fish and dairy products.

Comparing food portion sizes between individual and shared-plate feeding episodes, for most of food groups examined, we found, that children also consumed larger portion sizes if eating from a shared plate (Table 3). Portion sizes for grains were more than 1.5 times larger for children in shared-plate situations than if they ate individually. In the case of non-carotenoid–rich vegetables, pulses, non-carotenoid–rich fruits and dairy products, eating from a shared plate increased the portion size by nearly one third. There were no significant differences in portion sizes between cases and controls for any food group examined.

85

TABLE 2

Odds of consuming different food groups during shared-plate relative to individual-plate feeding episodes for all study children[1]

Food group	OR,[2] 95% CI Shared plate vs. individual plate
Grains[3]	12.2 (7.9–18.8)
Vegetables	2.7 (2.3–3.2)
Carotenoid-rich vegetables	2.1 (1.7–2.6)
Pulses	2.4 (2.0–2.9)
Fruits	1.4 (1.1–1.3)
Carotenoid-rich fruits	1.3 (0.8–2.0)
Meats and/or fish	2.1 (1.6–2.7)
Dairy products	2.2 (1.8–2.8)

[1] Adjusted for household socioeconomic status, household case status, sex of focus child, sequence of the observation day and the feeding episode within the day.
[2] OR, odds ratio.
[3] See text for description of categorization of food groups.

Feeding patterns in case and control households. General feeding behaviors of focus children were nearly identical between case and control households (data not shown). The average number of feeding episodes per child per observation day was 3.9. At least one shared-plate eating episode was observed on 65% of all observation days. If eating from the same plate, on average, 2.6 people ate together. Of all feeding episodes, the focus child ate in a shared plate with another child (19% of all feeding episodes), with an adult (12%) and with both an adult and another child (7%) (these are nonexclusive groups). Because the demographic composition of the family can modify food-sharing behaviors, we examined but found no differences between case or control households with respect to the number of adults (>15 y of either sex, median = 2) or children (<15 y, median = 4) of either sex living in the household.

There were significant differences in the types of foods consumed during feeding episodes of case and control focus children (**Table 4**). Comparing individual-plate feeding episodes between case and control children, cases were more likely to consume non-carotenoid–rich vegetables (OR = 1.3, CI = 1.1–1.6) than control children. This significant difference was also found when all feeding episodes were compared. Cases were more likely to consume carotenoid-rich vegetables during individual-plate eating compared with controls; however, this difference was not seen for all feeding episodes.

On the other hand, case children were significantly less likely to have access to key vitamin A–rich foods including meats and fish (OR = 0.6, CI = 0.5–0.9) and dairy products (OR = 0.7, CI = 0.5–1.0), compared with control children over all feeding episodes and especially during shared-plate feeding. No significant differences were noted in the consumption of carotenoid-rich vegetables or fruits.

The age and sex of an individual food sharer may influence consumption patterns of children eating with them. We examined whether case and control children differed with respect to the identity of their food sharers (**Table 5**). Case children were significantly more likely to eat in a shared-plate situation with a male adult (age > 15 y) (OR = 1.7, CI = 1.0–2.8), whereas case children were significantly less likely to eat with a female adult (OR = 0.6, CI = 0.4–0.9) or a female child (OR = 0.6, CI = 0.4–1.0) compared with non-xerophthalmic control children. No significant differences were noted between case and control children sharing a plate with a male child.

Table 6 presents the average total portion size (in grams) for food groups consumed in a feeding episode by the focus child on the basis of their case status and the age and sex of at least one food sharer. No statistical differences in food portion sizes could be discerned between any age-sex food-sharer

TABLE 3

Average total food portion size consumed per feeding episode of study children, by food group, plate-eating status and case status[1,2]

Food group	Case status	Individual plate (n = 3178)	Shared plate (n = 1099)	P value[3]
		g/feeding episode		
Grains[4]	Case	176 ± 12	273 ± 13	<0.001
	Control	163 ± 11	263 ± 13	
Vegetables	Case	60 ± 9	81 ± 9	<0.001
	Control	57 ± 8	66 ± 9	
Carotenoid-rich vegetables	Case	32 ± 13	34 ± 14	NS
	Control	37 ± 13	37 ± 13	
Pulses	Case	108 ± 17	122 ± 18	<0.05
	Control	110 ± 16	142 ± 17	
Fruits	Case	32 ± 26	27 ± 28	<0.001
	Control	16 ± 27	59 ± 27	
Carotenoid-rich fruits	Case	72 ± 28	65 ± 35	NS
	Control	63 ± 26	48 ± 33	
Meats and/or fish	Case	82 ± 14	69 ± 16	NS
	Control	59 ± 13	58 ± 14	
Dairy products	Case	85 ± 17	105 ± 18	<0.05
	Control	87 ± 16	116 ± 17	

[1] Values are means ± SD.
[2] Adjusted for household socioeconomic status and sex of focus child.
[3] Test for differences in least-square means of portions sizes between individual vs. shared-plate feeding episodes. NS, not significant.
[4] See text for description of categorization of food groups.

86

TABLE 4

Odds of consuming different food groups, by type of feeding episode for case relative to control focus children[1]

Food group	OR,[2] 95% CI case vs. Control		
	Individual plate	Shared plate	All feeding episodes
Grains[3]	1.1 (0.9–1.4)	1.2 (0.6–2.4)	1.1 (0.9–1.4)
Vegetables	1.3 (1.1–1.6)	1.0 (0.8–1.4)	1.2 (1.0–1.4)
Carotenoid-rich vegetables	1.4 (1.0–1.9)	0.8 (0.6–1.1)	1.1 (0.8–1.4)
Pulses	1.0 (0.8–1.3)	0.7 (0.5–1.0)	0.9 (0.7–1.1)
Fruits	1.2 (0.9–1.7)	0.7 (0.5–1.1)	1.0 (0.8–1.3)
Carotenoid-rich fruits	0.9 (0.6–1.4)	0.8 (0.4–1.6)	0.8 (0.6–1.2)
Meats or fish	0.7 (0.5–1.1)	0.5 (0.3–0.8)	0.6 (0.5–0.9)
Dairy products	0.8 (0.6–1.2)	0.6 (0.4–0.9)	0.7 (0.5–1.0)

[1] All models are adjusted for household socioeconomic status, sex of focus child, and sequence of the observation day and the feeding episode within the day; combined model is also adjusted for single vs. shared plate.
[2] OR, odds ratio.
[3] See text for description of categorization of food groups.

group or between case and control children. Nonetheless, some trends can be observed. The total average portion sizes for vegetables and meats or fish consumed by case children appear to be larger than that of control children. On the other hand, portion sizes for carotenoid-rich vegetables and dairy products appear to larger for control compared with case children.

DISCUSSION

To our knowledge, this is the first study that has systematically examined shared-plate eating and has been able to compare directly shared and nonshared-plate eating of the same children over multiple observations. The fact that shared-plate eating occurs regularly throughout the year (65% of days observed) and infrequently within the day (25% of feeding episodes) provided us with a unique opportunity to explore the relative advantages and disadvantages of both feeding patterns in this high risk population.

Assessments of dietary intake are very difficult in regions in which shared-plate eating is common. We were able to explore in detail the dynamics of shared-plate eating with the use of continuous monitoring observations. This data collection technique permitted us to track the temporal sequence of events contributing to the shared-plate episode and to better characterize different types of shared-plate eating. In our sample, we found the predominant form of shared-plate eating consisted of two or more individuals being served simultaneously. The other identified patterns, such as post-served and multiple post-served sharing may be important because the time at which an individual comes to share food during a meal

TABLE 5

Odds of participating in a shared-plate feeding episodes with an adult or child, by sex and age of at least one food sharer, for case relative to control focus children[1]

Sex and age of food sharer	OR,[2] 95% CI case vs. control
Male adult (>15 y)	1.7 (1.0–2.8)
Female adult (>15 y)	0.6 (0.4–0.9)
Male child (≤15 y)	1.2 (0.8–2.0)
Female child (≤15 y)	0.6 (0.4–1.0)

[1] Adjusted for household socioeconomic status and sex of focus child.
[2] OR, odds ratio.

TABLE 6

Average total food portion size consumed during shared-plate feeding episodes of study children, by food group, case status and by age and sex of at least one food sharer[1,2]

Food group	Case status	Male adult (>15 y) n = 283	Female adult (>15 y) n = 340	Male child (≤15 y) n = 500	Female child (≤15 y) n = 509
		g/feeding episode			
Grains[3]	Case	323 ± 17	298 ± 18	315 ± 16	323 ± 16
	Control	294 ± 20	295 ± 17	319 ± 16	309 ± 16
Vegetables	Case	90 ± 11	88 ± 11	100 ± 11	91 ± 11
	Control	59 ± 13	65 ± 11	72 ± 11	65 ± 11
Carotenoid-rich vegetables	Case	27 ± 17	29 ± 18	17 ± 18	39 ± 17
	Control	66 ± 20	46 ± 18	41 ± 19	43 ± 16
Pulses	Case	103 ± 26	92 ± 27	118 ± 24	119 ± 24
	Control	106 ± 29	137 ± 23	167 ± 22	149 ± 21
Fruits	Case	46 ± 39	42 ± 36	36 ± 35	56 ± 48
	Control	50 ± 37	18 ± 37	77 ± 31	58 ± 32
Carotenoid-rich fruits	Case	50 ± 63	53 ± 63	39 ± 66	68 ± 52
	Control	83 ± 72	109 ± 61	38 ± 46	70 ± 49
Meats and/or fish	Case	84 ± 33	72 ± 35	81 ± 33	67 ± 32
	Control	58 ± 32	54 ± 27	66 ± 26	56 ± 27
Dairy products	Case	83 ± 27	86 ± 28	101 ± 25	105 ± 22
	Control	121 ± 23	128 ± 22	135 ± 23	113 ± 20

[1] Values are means ± SD.
[2] Adjusted for household socioeconomic status and sex of focus child.
[3] See text for description of categorization of food groups.

87

may affect the total portion size that the individual receives. We were not able to discern such differences between sharing types because of the infrequency of the last-mentioned types of sharing in our sample.

There are a number of aspects of shared-plate eating that influence food consumption for children. Taken as a whole, we found shared-plate eating to be a positive behavior in that more and different types of foods were consumed. It is clear from our sample that shared-plate eating occurs mainly during meals within the household and very infrequently during snacks or meals that were consumed outside the home. As a result, it is not surprising that we found shared-plate feeding episodes were more likely to include a greater variety of food groups compared with other feeding episodes. In general, children eating on a shared plate were twice as likely to receive a specific food group than if they ate alone.

Food portion sizes consumed by young children during a shared-plate meal were also larger. Again, this may be partially due to the fact that individual-plate feeding episodes included snacks. However, the very large differences in portion size would suggest that, on average, one consumes more if eating with others than by oneself. This may be because the child has increased access to foods as it may be obtained or requested by a food sharer more senior than the child or simply a result of more than one individual requesting food. It should be noted, however, that despite the larger portion sizes obtained during a shared-plate feeding episode, the average portion sizes, especially for vegetables and meats, were still small.

These data suggest that shared-plate eating is a beneficial feeding behavior for young children, but the role it plays with respect to vitamin A deficiency is still unclear. Our study populations (cases and controls) were surprisingly similar in their basic feeding patterns. From earlier ethnographic work, we found that adults usually ate two meals (morning and evening) during the day and one snack (usually late afternoon). The children in our study appear to have eaten more frequently, having, on average, three meals and one snack each day. The frequency of shared-plate eating did not differ substantially between cases and controls.

The primary difference between the two groups was that, on a per meal basis, case children were significantly less likely to have any dairy products or meat and/or fish than control children. On the contrary, they were significantly more likely to consume non-carotenoid–rich vegetables, presumably as substitution for other foods. These data are consistent with feeding patterns identified using weekly food frequencies (Shankar et al. 1996) and infant diet histories (Gittelsohn et al. 1997) in the same population.

The identity of the person sharing food with the child appears to play a key role. Children having a history of xerophthalmia were significantly more likely to be found eating with a male adult, whereas similar aged non-xerophthalmic children were more likely to eat with a female adult or a female child ($\leq$15 y). On the basis of the literature on intrahousehold food distribution, this would suggest that non-xerophthalmic children are benefiting from the increased child attentiveness provided by the female adult (Rogers and Schlossman 1990). There is evidence that women, especially mothers, are more likely to encourage a child to eat than other caregivers (Engle and Zeitlin 1996) and are more attentive during feeding when a child is ill (Bentley 1988, Bentley et al. 1991). Even as young children, girls are found to offer and ask for food more than boys and are more likely to share their food with others (Dyson-Hudson and Van Dusen 1972).

Studies examining the effectiveness of male interactions with respect to child rearing indicate that although men can parent, they tend not to be as responsive as women to the child's needs (Mackey 1983). Therefore in a shared-plate setting with a male adult, the case child may be neglected or may otherwise procure less food. This is balanced by the fact that, in this setting, male adults, because of their high social status, may be the recipients of "higher quality" foods (Gittelsohn, 1991), which may increase a young child's access to these foods.

In our examination of food portion sizes, we found little evidence that the sex and age of the food sharer significantly influenced the total portion size of consumed foods. This may be due, in part, to the variability of the portion size estimates. These identified patterns in shared-plate eating may reflect the overall poorer care experienced by children with a history of xerophthalmia. In earlier analyses, we found that case children were less likely to be groomed (A. V. Shankar, unpublished manuscript), more likely to experience negative social behaviors, such as hitting and scolding, and less likely to have positive health behaviors, such as face cleaning or using soap than control children (Gittelsohn, et al. 1998). It is likely that eating with an adult male is a risk factor for vitamin A deficiency only in that it is characteristic of households in which poor child caretaking exists.

This study has examined the very common, but poorly researched phenomenon of shared-plate eating. With the use of continuous monitoring observation, we were able to identify the temporal sequence, actors and recipients of dietary behaviors as well as approximate the total amount of food consumed by each child and posit a relationship between these feeding patterns and nutritional status. The shared-plate eating patterns we have identified are slightly different than those reported in the literature. Shared-plate eating is not the primary method of food consumption in this population and children often eat only with other young children, not with adults. It is likely that this type of food sharing occurs in other areas of southern Nepal and northern India, although it may be evident in other regions of South Asia as well.

There are still many gaps in our knowledge with respect to food sharing. For example, how does illness affect total portion size consumed especially because a child's appetite plays a large role in food intake (Engle and Zeitlin 1996)? What are the specific behavioral mechanisms that occur in a shared-plate setting that assist the child to eat more? Can these behaviors be taught to other household members? Because food sharing from a single bowl or plate is so common in developing countries, shared-plate dynamics must be evaluated in different settings to identify their potential effect on dietary outcomes.

ACKNOWLEDGMENTS

The study group in Sarlahi (besides authors) includes Subarna Khatry, Steven LeClerq, Elizabeth K. Pradhan, Sharada Ram Shrestha, and NoorNath Acharya and Ram Prasad Pokhrel. The results presented here are part of a collaborative effort among The Johns Hopkins University, Baltimore, MD, Nepal Netra Jyoti Sangh (National Society for the Prevention of Blindness) and the Nepal Eye Hospital in Nepal. The authors acknowledge the contributions of Elizabeth Montgomery in the preparation of this manuscript. The authors express their sincere appreciation to all field observers, Ram Asheswar Chaudhary, Ram Chandra Chaudhary, Keshab Dhakal, Rajendra Dhami, Anil Karna, Yadav Mainali, Khadka Ray, Mukunda Shrestha, Hira Thapaliya, Jageshwar Dhami Tharu and the DIVA data entry team in Kathmandu, managed by Elizabeth Pradhan and Sunita Pant, for their hard work.

LITERATURE CITED

Abdullah, M. & Wheeler, E. (1985) Seasonal variations, and the intra-household distribution of food in a Bangladeshi village. Am. J. Clin. Nutr. 41: 1305–1313.

88

Basu, A., Roy, S. K., Mukhopadhyay, B., Bharati, P., Gupta, R. & Majumder, P. P. (1986) Sex bias in interhousehold food distribution: roles of ethnicity and socio-economic characteristics. Curr. Anthropol. 27: 536–539.

Beckerleg, S. (1995) Food bowl division and social change. Ecol. Food Nutr. 34: 89–104.

Bentley, M. E. (1988) The household management of childhood diarrhea in rural North India. Soc. Sci. Med. 27: 75–85.

Bentley, M. E., Boot, M. T., Gittelsohn, J. & Stallings, R. (1994) The use of structured observations in the study of health behavior, Occasional Paper 27. International Reference Centre, International Water and Sanitation Centre, The Hague, The Netherlands.

Bentley, M. E., Stallings, R. Y., Fukumoto, M. & Elder, J. A. (1991) Maternal feeding behavior and child acceptance of food during diarrhea, convalescence, and health in the Central Sierra of Peru. Am. J. Public Health 81: 43–47.

Blurton Jones, N. G. (1984) A selfish origin for human food sharing: tolerated theft. Ethnol. Sociobiol. 5: 1–3.

Carloni, A. S. (1981) Sex disparities in the distribution of food within rural household. Food Nutr. Bull. 7: 3–12.

Chen, L. C., Huq, E. & D'Souza, S. (1981) Sex bias in the family allocation of food and health care in rural Bangladesh. Pop. Dev. Rev. 7: 55–70.

Dettwyler, K. A. (1989) Styles of infant feeding: parental/caretaker control of food consumption in young children. Am. Anthropol. 9: 696–703.

Dyson-Hudson, R. & Van Dusen, R. (1972) Food sharing among young children. Ecol. Food Nutr. 1: 319–324.

Engle, P. L. (1990) Intra-household allocation of resources: perspectives from psychology. In: Intra-Household Resource Allocation (Rogers, B. L. & Schlossman, N. P., eds.), pp. 63–82. UNU University, Tokyo, Japan.

Engle, P. & Nieves, I. (1993) Intra-household food distribution among Guatemalan families in a supplementary feeding program: behavior patterns. Soc. Sci. Med. 36: 1605–1612.

Engle, P. L. & Zeitlin, M. (1996) Active feeding behavior compensates for low interest in food among young Nicaraguan children. J. Nutr. 126: 1808–1816.

Farb, P. & Armelogos, G. (1980) Consuming Passions—the Anthropology of eating. Houghton Mifflin Company, Boston, MA.

Feinman, S. (1979) An evolutionary theory of food sharing. Soc. Sci. Inform. 18: 695–726.

Gittelsohn, J. (1989) Intrahousehold Food Distribution in Rural Nepal. Doctoral thesis, University of Connecticut, Storrs, CT.

Gittelsohn, J. (1991) Opening the box: intrahousehold food allocation in rural Nepal. Soc. Sci. Med. 33: 1141–1154.

Gittelsohn, J., Shankar, A. V., Pokhrel, R. P. & West, K. P., Jr. (1994) Accuracy of estimating food intake by observation. J. Am. Diet. Assoc. 94: 1273–1277.

Gittelsohn, J., Shankar, A. V., West, K. P. Jr., Ram, R., Dhungel, C. & Dahal, B. (1997) Infant feeding history reflects antecedent risk of xerophthalmia in Nepali children. Eur. J. Clin. Nutr. 51: 484–490.

Gittelsohn, J., Shankar, A. V., West, K. P., Jr., Faruque, F., Ghywali, T. & Pradhan, E. (1998) Child feeding and care behaviors are associated with xerophthalmia in rural Nepal. Soc. Sci. Med. (in press).

Goldschmidt, W. (1966) Comparative Functionalism. University of California Press, Berkeley, CA.

Harriss, B. (1990) Food distribution, death and disease in South Asia. In: Diet and Disease (Harrison, G. A. & Waterlow, J. C., eds.), pp. 137–154. Cambridge University Press, Cambridge, UK.

Hudson, G. (1995) Food intake in a West African village. Estimation of food intake from a shared bowl. Br. J. Nutr. 73: 551–569.

Mackey, W. C. (1983) A preliminary test for the validation of the adult male-child bond as a species characteristics trait. Am. Anthropol. 85: 391–402.

Panter-Brick, C. (1995) Food and household status in Nepal. In: Food and the Status Quest (Weissner, P. & Schiefenhovel, W., eds.). Berghahn Books, Oxford, UK.

Pelto, G. (1984) Intrahousehold food distribution patterns. In: Malnutrition: Determinants and Consequences (Davis, G., ed.), pp. 285–293. Alan R. Liss, New York, NY.

Rogers, B. L. & Schlossman, N. P., eds. (1990) Intra-Household Resource Allocation: Issues and Methods for Development Policy and Planning. United Nations University Press, Tokyo, Japan.

SAS Institute Inc. (1995) Release 6.12. SAS Institute, Cary, NC.

Shankar, A. V. (1995) In Search of the Light in Your Eyes: Anthropological and Epidemiological Perspectives of Vitamin A Deficiency in Nepali Children. Doctoral thesis, Case Western Reserve University, Cleveland, OH.

Shankar, A. V., West, K. P. Jr., Gittelsohn, J., Katz, J. & Pradhan, R. (1996) Chronic low intakes of vitamin A-rich foods in households with xerophthalmic children: a case-control study in Nepal. Am. J. Clin. Nutr. 64: 242–248.

Sommer, A. (1982) Nutritional Blindness: Xerophthalmia and Keratomalacia. Oxford University Press, New York, NY.

Van Esterick, P. (1985) Intra-family food distribution: its relevance for maternal and child nutrition. In: Determinants of Young Child Feeding and Their Implications for Nutritional Surveillance. Cornell Nutritional Surveillance Program Working Paper No. 31, Cornell University, Ithaca, NY.

Zeger, S. L., Liang, K. Y. & Albert, P. S. (1988) Models for longitudinal data: a generalized estimating equation approach. Biometrics. 44: 1049–1060.

Home gardening and access to animals in households with xerophthalmic children in rural Nepal

Anita V. Shankar, Joel Gittelsohn, Elizabeth K. Pradhan, Chandra Dhungel, and Keith P. West, Jr.

Abstract

This case–control study compares the home garden and animal husbandry practices of households with and without xerophthalmic children in south-central Nepal, focusing on the relationship between these practices and household intake of vitamin A–rich foods. Eighty-one households with a child between the ages of one and six years diagnosed with xerophthalmia (cases) and 81 households with an age-matched, non-xerophthalmic child (controls) were studied. There was little difference between case and control households in the size of their gardens. However, case households were significantly less likely to plant carotenoid-rich vegetables from October to March than were control households (odds ratio, 0.39; 95% confidence interval, 0.16 to 0.96). The mean consumption of non-carotenoid-rich vegetables, but not of carotenoid-rich vegetables, increased linearly with garden size. Case households were significantly more likely than control households to rent domesticated animals from others ($\chi^2 = 5.91$; $p < .05$). Control households were more likely than case households to own chickens and pigeons ($\chi^2 = 6.6–9.2$; $p < .05$). During specific seasons, household meat consumption was significantly lower in case households, regardless of access to animals. Case households appeared to have significantly lower intakes of key vitamin A–rich foods, particularly green leaves and meat, regardless of their socio-economic level (as determined by ownership of material goods), access to animals, or availability of home gardens.

Anita V. Shankar, Joel Gittelsohn, Elizabeth K. Pradhan, and Keith P. West, Jr., are affiliated with the Division of Human Nutrition, Department of International Health, in the School of Hygiene and Public Health at the Johns Hopkins University in Baltimore, Maryland, USA. Chandra Dhungel is affiliated with the Nepal Nutrition Intervention Project-Sarlahi in Kathmandu, Nepal.

Mention of the names of firms and commercial products does not imply endorsement by the United Nations University.

Introduction

In areas where vitamin A deficiency is a serious public health problem, childhood xerophthalmia is known to cluster at the regional, district, village, and household levels [1]. This clustering is thought to be the result of pockets of relative poverty and/or poor access to vitamin A–rich food sources [2–5]. Despite this, there are a number of households living in these same high-risk clusters that do not experience xerophthalmia. One explanation is that these households have developed strategies that allow greater access to vitamin A–rich foods.

Cultivation of home gardens and ownership of domesticated animals are potential ways of increasing household access to foods rich in vitamin A and carotenoids. Numerous attempts have been made to implement gardening programmes towards such a goal [6, 7]. Indeed, several studies have found that the presence of a home garden is a strong predictor of the quality of child nutrition, particularly during seasons when employment opportunities are few [8–10].

Although home gardens have been shown to provide a number of economic and dietary advantages to poor families, the evidence linking home gardens to reduced vitamin A deficiency in vulnerable household members is equivocal. In Bangladesh, analyses indicate that even a small household garden affords protection against childhood xerophthalmia [4]. More recent work from this region [11] shows that the variety of plants in a garden, and not its size, is associated with reduced risk of xerophthalmia. Other studies, however, show that vitamin A status may not improve despite successful home gardening efforts [12, 13]. In West Java, well-off households (those with home gardens and land) were found to consume more of all nutrients than did poorer households, except for vitamin A and calcium [14].

Household-level interventions directed towards improving vitamin A status have focused on increasing plant sources of vitamin A by encouraging home gardens, which require a relatively small investment and can be a source of abundant carotenoid-rich foods. Domestic animals are an additional source of home

90

food that intervention studies and programmes often overlook. Domestic animals provide milk, eggs, and meat that may help protect household members from vitamin A deficiency.

In this study we explore the variations in access to domesticated animals and differences in home gardening practices in households with and without xerophthalmic children in rural Nepal. These practices are then examined with respect to the changes in intake of carotenoid- and retinol-rich foods at the household level over a one-year period.

Methods

A community-wide ocular survey was conducted in January 1992 on 3,735 pre-school children living in three Village Development Committees with a population of approximately 15,000 in Sarlahi District in the central lowland region of Nepal. The survey identified 86 households with children aged 12 to 72 months who had clinical signs of xerophthalmia or a current history of night-blindness. Five households were lost because of migration or refusal to participate, resulting in 81 case households participating in the study. Thirty-three of the children had guardian-reported night-blindness alone, 33 had Bitot's spots alone, and 10 children had both night-blindness and Bitot's spots. The remaining five were diagnosed with corneal xerosis alone or with Bitot's spots or night-blindness.

From the pool of neighbouring households (within 5 km of the case), 81 control households were selected that had a child of the same age as the case child (within three months) and a younger sibling of similar age (within four months), if a younger sibling was present in the case household. In control households, there were no clinical signs of xerophthalmia in any child in the household and no reported history of night-blindness in children or their mothers. Both case and control households were studied prospectively from March 1993 to June 1994.

A recall of home gardening practices was obtained from the head of the household, usually a male, three times during the year: April–May 1993 (three-month recall period), August–September 1993 (four-month recall period), and February–March 1994 (five-month recall period). For the purposes of this study, a home garden was defined to include all plants (fruits and vegetables) grown in and around the homestead. We used specific local terms to distinguish home gardens from agricultural plots. During each round of interviews, recall data were collected on which vegetable and fruit plants were grown in the home garden, the quantity grown, and about how much of the produce was consumed in the household. Harvesting and consumption of green leaves associated with gourds (such as pumpkin leaves) were also noted.

The size of the vegetable garden was calculated by summing the total plot sizes used for each type of plant grown. If one plot area was used for two different crops (planted simultaneously or at different times), the total garden size equalled twice the actual land area used by the plants. In addition, individual vines and plants, such as gourds and tomatoes, grown outside a formal plot were counted separately and assigned an average value of 0.5 square feet (464 cm^2) per plant. As a result of varying planting and harvesting intervals during the recall period, the calculation of garden size is not representative of the actual amount of cultivated land.

Only fruit plants and trees that produced fruit during the year surveyed were counted. The informants were asked to recall all vegetables and fruit plants to which their family had access and from which they were able to obtain some produce, such as plants shared with relatives or neighbours. Fruits or leaves found in the wild were not included within our definition of home garden.

Possession of domesticated animals was determined once by a survey conducted with the head of the household in January 1993. In Nepal there are several forms of animal ownership, ranging from outright ownership to renting and raising animals for a pre-determined portion of the meat or milk produced. Ownership of an animal, for this study, means that the owner has access to all milk or eggs produced by the animal and all the meat if the animal is slaughtered. Rental of an animal means that part of the animal products are kept by the owner and part by the household renting the animal. The numbers of animals owned, rented from others, and rented to others were determined for each household.

Household dietary intake was assessed with a previously described seven-day food-frequency questionnaire [15]. The food-frequency questionnaire was administered four times during the year beginning in July 1993: July to September, October to December, January to March, and April to June. Dietary recalls were conducted on individuals knowledgeable about the household and the food intake of the children, usually the mothers. Household intake was defined as foods consumed by any member of the household, focusing on foods consumed during the primary mealtimes.

Fruits and vegetables were classified as carotenoid-rich or non-carotenoid-rich on the basis of regional and international food composition tables [16, 17]. Vegetables classified as carotenoid-rich included dark-green leafy vegetables and ripe pumpkin; fruits classified as carotenoid-rich included papayas, mangoes, and jackfruit.

Matched-pair odds ratios and 95% confidence intervals were derived by conditional logistic regression to compare household characteristics according to xerophthalmia status. All odds ratios were adjusted for socio-economic status, which was a scaled variable based on the number of different types of material possessions not related to either home gardening efforts or the up-

keep of animals. The final scale had eight items in currently usable condition: flashlights (one or more), watches (one or more), bicycles (one or more), armoires (one or more), beds (two or more), number of rooms in the house (two or more), a roof constructed of a material other than straw, and irrigation pump sets. The alpha coefficient for this scale was 0.78 and the mean score value was 3. Those households with a scale score equal to or greater than the mean were classified as having higher socio-economic status (a value of 1 in the regression equation); those below the mean were classified as having lower socio-economic status (a value of 0).

Odds ratios (OR) of less than 1 indicate that case households were less likely than controls to report a practice (e.g., intake of foods). For analyses that required further stratification of the data, unmatched-pair analyses were conducted. Analyses were carried out using SAS (SAS version 6.11, SAS Institute, Cary, NC, USA).

Oral informed consent was obtained from families before participation in the study. This study was approved by the Nepal Health Research Council in Kathmandu and the Committee for Human Research, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, USA.

Results

Household gardening patterns

Table 1 lists the most common fruits and vegetables grown over the year by case and control households. The types of vegetables and fruits planted and picked differ little between the two groups. An exception, however, is *munga*, a type of long thin gourd, which was planted significantly more frequently in control households. No difference was noted in access to gardens: more than 97% of all study households reported access to some type of home garden during the year, approximately 95% (97% cases, 94% controls) of the households cultivated vegetables, and more than 81% (80% cases, 84% controls) produced fruits. Likewise, no difference was found in the percentage (which averaged 60%) of vegetable and fruit harvests that the household reported were completely consumed.

Garden size varied during the year, with the smallest gardens being planted from June to September and the largest from October to March. Table 2 compares case and control households with respect to garden size and composition according to season. There were no significant differences in garden size between case and

TABLE 1. Percentage of households with access to garden vegetables and fruits during the year, according to case status

Common name	Scientific name	Cases ($n = 79$)	Controls ($n = 77$)
Vegetables			
Sponge gourd	*Luffa cylindrica*	84	75
Green chilies	*Capsicum annum grossa*	72	64
Green peas	*Pisum sativum*	66	64
Gourd	*Lagemaria siceraria*	66	62
Potato	*Impomea batas-convulacea*	58	69
Pointed gourd	*Trichosonthes dioica*	58	66
Munga	Gourd, like *Moringa oleifera*	37	55[a]
Eggplant	*Solanum melongena-solonacea*	33	34
Carotenoid-rich vegetables			
Pumpkin	*Cucurbita minima*	68	77
Garlic leaves	*Allium sativum*	57	65
Mustard leaves	*Brassica compestris-cruciferae*	46	51
Dhaniya leaves	*Coriander sativum*	32	34
Thadiya leaves	Name unknown	32	23
Radish leaves	*Raphnum sativus-crucifera*	25	32
Fruits			
Banana	*Musa sapientum*	52	53
White guava	*Psidium guajava*	57	49
Custard apple	*Annona squamosa*	23	28
Carotenoid-rich fruits			
Papaya	*Carica papaya*	71	65
Mango	*Mangifera indica*	52	56
Jackfruit	*Artocarpus integrifolia*	48	49

a. Statistically significant at $p < .05$.

92

control households over the whole year; however, control households tended to have larger gardens than case households from October to March (OR, 0.41; 95% CI, 0.17 to 1.01). From October to March only, control households were significantly more likely than case households to plant and consume at least one carotenoid-rich vegetable (OR, 0.39; 95% CI, 0.16 to 0.96). This difference is mainly a result of the greater number of garlic leaves and onion greens consumed in control households during this period.

Figure 1 shows the relationship between average cumulative garden size and vegetable consumption for the year for both vitamin A–rich foods and those lacking significant amounts of vitamin A. Pooled data were used for these comparisons, as garden size did not significantly differ between cases and controls. Consumption of non–vitamin A–rich vegetables was found to increase with garden size, whereas consumption of vitamin A–rich vegetables remained low across the observed range of garden sizes. A similar analysis was performed for fruit consumption (fig. 2). In this case, households without gardens had roughly half the mean intake of carotenoid-rich fruits as households with large gardens, although this difference was not statistically significant.

Patterns of animal husbandry

Table 3 compares case and control households with respect to their access to domesticated animals. Nearly all households (90% of cases and 95% of controls) had at least one animal in their home, and nearly 50% of case and control households had a milk-producing animal. However, case households were more likely than controls to have rented animals from others, especially cows. Moreover, nearly twice as many control households as case households had chickens, and three times as many control households as case households had

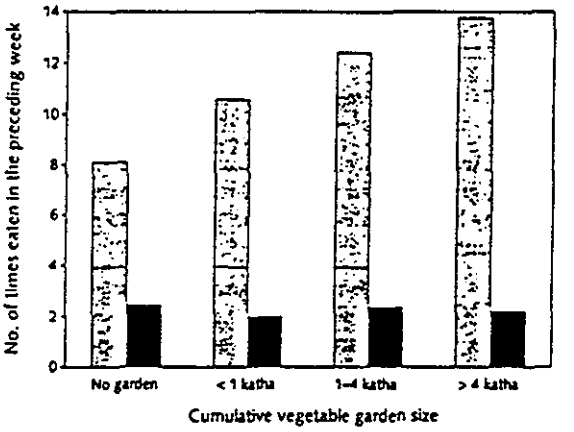


FIG. 1. Average weekly household consumption of low-carotenoid (grey bars) and carotenoid-rich (black bars) vegetables by all study households over the year, according to size of vegetable garden. 1 katha = 0.083 acres = 0.034 hectares

TABLE 2. Matched-pair adjusted odds ratios of garden characteristics for case and control households

Garden characteristic	Feb–May 1993			Jun–Sep 1993			Oct 1993–Mar 1994		
	Case–control pairs ($n = 78$) (%)	OR[a]	95% CI	Case–control pairs ($n = 78$) (%)	OR	95% CI	Case–control pairs ($n = 77$) (%)	OR	95% CI
Cumulative plot size[b] (> 1 katha of land)	63 59	1.49	0.74–3.02	22 16	1.80	0.75–4.32	58 71	0.41	0.17–1.01
> 10 single vines, bushes	44 49	0.84	0.44–1.61	62 58	1.27	0.62–2.62	43 46	0.95	0.47–1.94
> 10 harvesting fruit plants	17 21	0.86	0.36–2.02	33 41	0.87	0.45–1.69	22 36	0.67	0.33–1.35
Carotenoid-rich vegetables harvested and consumed in household	64 71	0.82	0.41–1.65	24 27	0.92	0.45–1.92	72 87	0.39	0.16–0.96[c]
Carotenoid-rich fruits harvested and consumed in household	39 45	0.82	0.43–1.57	39 45	0.70	0.36–1.39	47 42	1.40	0.74–2.65

a. OR, odds ratio; OR < 1 indicates that case households are less likely than control households to report a practice.
b. 1 katha = 0.083 acres = 0.034 hectares. Garden size includes calculation of single vines and bushes with designated plot size of 0.5 sq. ft. (464 cm^2) per plant.
c. $p < .05$.

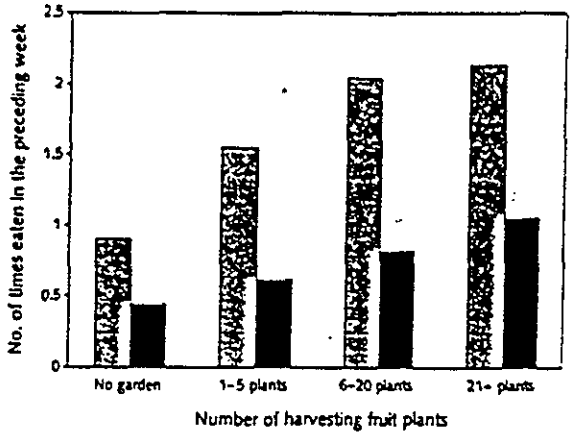


FIG. 2. Average weekly household consumption of low-carotenoid (grey bars) and carotenoid-rich (black bars) fruits by all study households over the year, according to number of fruit plants

pigeons. Chickens and pigeons are both good sources of vitamin A–rich eggs and meat, including liver.

Using household food-frequency data, table 4 examines differences in milk consumption by any household member in the previous week, stratified by access to milk-producing animals. Comparisons are made for three groups: (1) households with no milk-producing animals, (2) households with milk-producing animals (either rented or owned), and (3) households that owned but did not rent milk-producing animals (a subset of group 2). Preliminary unadjusted odds ratios indicated that regardless of access to milk-producing animals (rented or owned), case households were significantly less likely than control households to have consumed milk during the periods from July to September and from October to December (odds ratios ranged from 0.16 to 0.33). However, adjustment for socio-economic status removed any significant differences in milk consumption between case and control households.

Meat (including liver) and eggs are also good sources of pre-formed vitamin A. Animals most likely to be domesticated for consumption include chickens, pigs, goats, and pigeons. Table 5 compares case and control households with and without access to these animals with respect to their consumption of meat and eggs. Among households without access to animals, case households were much less likely to report consumption of meat or eggs in the preceding week than controls (odds ratios ranged from 0.07 to 0.69), with significant differences during the period from January to March. Among households with access to animals, case households were significantly less likely than controls to report consumption of meat or eggs in the preceding week during the period from October to December (OR, 0.41; 95% CI, 0.20 to 0.82).

Discussion

In this paper we differentiate households with a xerophthalmic child from those having a non-xerophthalmic child of the same age, with respect to two factors that could influence the intake of vitamin A–rich foods: home gardens and access to domesticated animals. After controlling for socio-economic status and season, the presence or absence of these factors was related to household intake of vitamin A–rich foods.

Previous dietary analyses from this study indicated that control households consumed more carotenoid-rich vegetables from July to December than case households [15]. The current data corroborate previous find-

TABLE 3. Percentage of case (n = 81) and control (n = 81) households raising animals, renting animals from others, or renting animals to others

Animal	Raising animals		Renting animals from others		Renting animals to others	
	Case	Control	Case	Control	Case	Control
Cows	25	26	11	0[a]	3	7
Bulls and calves	56	58	10	7	3	4
Buffaloes (female)	6	9	0	0	0	0
Buffaloes (males and calves)	21	35	9	9	3	5
Goats (female)	24	25	4	4	1	0
Goats (males and kids)	56	58	23	20	5	12
Pigs	4	6	2	0	0	2
Chickens	26	46[a]	6	5	1	4
Pigeons	6	20[a]	0	0	0	0
Any milk-producing animal (cows, buffaloes, goats)	48	47	15	4[a]	4	7
Any animal	90	95	43	31	11	24[a]

a. $p < .05$ (unmatched pair analyses).

94

TABLE 4. Non-adjusted and adjusted[a] odds ratios for milk consumption (at least once per household in the past week), based on access to milk-producing animals,[b] for cases and controls, according to year and months

Year and months	No access		Access[c]		Owned animals	
	OR	95% CI	OR	95% CI	OR	95% CI
1993 Jul–Sep						
Non-adjusted	1.25	0.48–3.23	0.32	0.12–0.80[d]	0.33	0.12–0.89[d]
Adjusted	1.07	0.45–2.51	0.73	0.34–1.57	0.61	0.26–1.43
1993 Oct–Dec						
Non-adjusted	1.56	0.62–3.97	0.16	0.06–0.45[d]	0.21	0.07–0.61[d]
Adjusted	1.30	0.53–3.15	0.57	0.26–1.26	0.55	0.24–1.28
1994 Jan–Mar						
Non-adjusted	1.10	0.37–3.30	0.41	0.16–1.02	0.46	0.17–1.23
Adjusted	0.96	0.33–2.78	0.79	0.33–1.90	0.82	0.31–2.15
1994 Apr–Jun						
Non-adjusted	0.47	0.17–1.26	0.69	0.28–1.71	0.90	0.34–2.39
Adjusted	0.41	0.15–1.12	1.41	0.58–3.43	1.78	0.63–5.06

a. Odds ratios adjusted for socio-economic level as determined by a scaled variable of material possessions owned.
b. Female cows, buffaloes, and goats.
c. Households raising animals, either owned or rented.
d. $p < .05$.

ings by showing that control households were more likely to consume carotenoid-rich vegetables from their gardens from October to March as well. The increased intake of carotenoid-rich foods found in the previous analyses may be due in part to the greater access to garden vegetables during certain months of the year.

TABLE 5. Non-adjusted and adjusted[a] ratios for consumption of meat or eggs (at least once per household in the past week), based on access to commonly used animals,[b] for cases and controls, according to year and months

Year and months	No access		Access[c]	
	OR	95% CI	OR	95% CI
1993 Jul–Sep				
Non-adjusted	0.42	0.08–2.33	0.60	0.28–1.26
Adjusted	0.69	0.14–3.29	0.69	0.34–1.38
1993 Oct–Dec				
Non-adjusted	0.19	0.03–1.11	0.31	0.14–0.67[d]
Adjusted	0.34	0.06–1.92	0.41	0.20–0.82[d]
1994 Jan–Mar				
Non-adjusted	0.07	0.01–0.47[d]	0.70	0.32–1.52
Adjusted	0.08	0.01–0.91[d]	0.77	0.37–1.63
1994 Apr–Jun				
Non-adjusted	0.39	0.56–2.73	0.71	0.35–1.48
Adjusted	0.65	0.11–3.91	0.80	0.42–1.51

a. Odds ratios adjusted for socio-economic level as determined by a scaled variable of material possessions owned.
b. Pigeons, chickens, pigs, and goats.
c. Households raising animals, either owned or rented.
d. $p < .05$.

The likelihood of improving consumption of vitamin A–rich foods from the garden, however, is not dependent on garden size. Larger garden sizes were not found to be associated with reported intake of dark-green leafy vegetables and pumpkin. This result supports those of other studies [8, 14] showing that higher economic status may not result in greater consumption of carotenoid-rich vegetables. On the other hand, there was a corresponding increase in the consumption of non-carotenoid-rich vegetables with garden size. As in other areas of South Asia, in Nepal dark-green leafy vegetables are often considered low-status foods, which may explain, in part, why increasing the areas of home gardens does not increase the number of times carotenoid-rich vegetables are eaten.

In this population, access to gardens was high and did not differ significantly between case and control households. Nevertheless, the mean consumption of carotenoid-rich fruits and vegetables was extremely low, averaging less than four times in the preceding week. We also found an increase in the consumption of all fruits as garden size increased. Additional efforts to promote home gardens will likely need to focus on greatly increasing the number of types of carotenoid-rich foods planted and consumed in the household.

Access to domesticated animals is another household strategy that may increase vitamin A intake at the household level but is often omitted from the home-gardening literature. In our sample, both case and control households had access to some type of domesticated animal. However, the form of ownership varied: case households were more likely to rent animals than to own

95

them. In addition, control households were significantly more likely than case households to own chickens and pigeons. This may be indicative of the slightly better socio-economic status of the control households, a finding consistent with other research in the area [3].

In an effort to control for the effects of socio-economic status on diet, we devised a socio-economic status scale that could serve as a proxy for household income but would not include items on ownership of land or animals. When this variable was included in the regression analysis, we found that socio-economic status did influence the odds ratios of household milk consumption but not those of consumption of meat and eggs. In the case of milk consumption, the odds ratios adjusted for socio-economic status were higher (0.5 to 0.7) than the unadjusted odds ratios (0.2 to 0.3), which implies that although control households had greater reported milk intake than cases, this was probably due in part to the slightly higher economic status of these households.

This was not true with consumption of meat or eggs; case households consumed meat or eggs significantly less frequently than controls in certain seasons, regardless of access to animals or socio-economic status. In households with no access to animals, the odds ratios for consumption of meat or eggs were low (0.07 to 0.69), suggesting that control households, even those without direct access to meat or eggs at home, were still able to obtain and consume meat or eggs more often than case households.

As efforts continue to identify household strategies that increase the intake of vitamin A–rich foods [18, 19], understanding the intricacies of current household practices and their relationship to diet is critical. This study demonstrates that household-level access to home gardens or domesticated animals does not ensure that vulnerable members within the household will be able to obtain a diet adequate to prevent clinical signs of vitamin A deficiency. In addition, the size of a garden did not appear to be related to the intake of carotenoid-rich vegetables in this population. From previous analyses [15] we learned that case households and index children were significantly less likely to consume meat, liver, and eggs. This finding holds true in the current analysis, which indicates that other factors besides household access to domesticated animals or socio-economic level (as determined by ownership of material goods) may influence household intake during critical periods of the year. This study is a first step in examining household strategies and their effect on household intake. Further research to identify other types of household strategies and characteristics important for increased consumption of vitamin A–rich foods is warranted.

Acknowledgements

The study group in Sarlahi included the authors and Dr. Rajendra Pradhan, Dr. Subarna Khatry, Mr. Steven LeClerq, Mr. Sharada Ram Shrestha, Mr. NoorNath Acharya, and Dr. R. P. Pokhrel. The study is a collaborative effort between the Johns Hopkins University, Baltimore, Maryland, USA, the Nepal Netra Jyoti Sangh (National Society for the Prevention of Blindness), and the Nepal Eye Hospital. The authors express their sincere appreciation and thanks to the DIVA interviewers, Shishir Shrestha, Ramekwal Chaudhary, Sulochana Chaudhary, and Ishwari Shrestha. The project was funded through Cooperative Agreement No. DAN 0045-A-00-5094-00 between the Center for Human Nutrition/Dana Center for Preventive Ophthalmology of the Johns Hopkins University and the Office of Health and Nutrition of the US Agency for International Development, with additional support from Task Force Sight and Life (Roche, Basel, Switzerland) and National Institutes of Health shared instrument grant No. S10-RR 04060.

References

1. Katz J, Zeger SL, West KP, Tielsch JM, Sommer A. Clustering of xerophthalmia within households and villages. Int J Epidemiol 1993;22:709–15.
2. Mele L, West KP Jr, Kusdiono, Pandji A, Nendrawati H, Tilden RL, Tarwotjo I. Nutritional and household risk factors for xerophthalmia in Aceh, Indonesia: a case-control study. Am J Clin Nutr 1991;53:1460–5.
3. Khatry SK, West KP Jr, Katz J, LeClerq SC, Pradhan EK, Wuls Thapa MD, Pokhrel RP. Epidemiology of xerophthalmia in Nepal. Arch Ophthalmol 1995; 113:425–9.
4. Cohen N, Jalil MA, Rahman H, Matin MA, Sprague J, Islam J, Davison J. Landholding, wealth and risk of blinding malnutrition in rural Bangladeshi households. Soc Sci Med 1985;21:1269–72.
5. Sommer A, West KP Jr. Vitamin A deficiency: health, survival and vision. New York: Oxford University Press, 1996.
6. Swedberg EA, Stetson V, Adrian JM, Zayan A. Multiple approaches to vitamin A (abstract). Report of the XVI IVACG Meeting, Chiang Rai, Thailand. Washington, DC: Nutrition Foundation, 1996:92.
7. Talukder A, Bloem MW. Home gardening activities in Bangladesh. Dhaka: Helen Keller International, 1992.
8. Kumar SK. Role of the household economy in child nutrition at low-incomes: case study in Kerala. Occasional Paper No. 95. Ithaca, NY, USA: Department of Agricultural Economics, Cornell University, 1978.
9. Ensing B, Sangers S. Home gardening in a Sri Lankan wet zone village: Can it contribute to improved nutrition? Wageningen, Netherlands: Wageningen Agricultural University, 1986.
10. Immink MDC, Sanjur D, Colon M. Home gardens and the energy and nutrient intakes of women and pre-

schoolers in rural Puerto Rico. Ecol Food Nutr 1991; 11:191–9.
11. Talukder A, Islam N, Tabibul AK, Desilles S, Bloem MW. Results and lessons learned from Helen Keller International's home gardening activities in Bangladesh (abstract). Report of the XVI IVACG Meeting, Chiang Rai, Thailand. Washington, DC: Nutrition Foundation, 1994:92.
12. Smitasiri S. Nutri-action analysis: going beyond good people and adequate resources. Bangkok: Amarin Printing and Publishing Public Company, 1994.
13. Smitasiri S. Advocating a multidimensional evaluation approach for comprehensive nutrition communication programs. INMU Special Publication Series No. 1. Bangkok: Institute of Nutrition, Mahidol University, and UNICEF East Asia and Pacific Regional Office, 1994.
14. Abdoellah OS, Marten G. The complementary roles of home gardens, upland fields, and rice fields for meeting nutritional needs in West Java. In: Marten GG, ed. Traditional agriculture in South-east Asia: a human ecology perspective. Boulder, Col, USA: Westview, 1986:293–325.
15. Shankar AV, West KP Jr, Gittelsohn J, Katz J, Pradhan R. Chronic low intakes of vitamin A–rich foods in households with xerophthalmic children: a case-control study in Nepal. Am J Clin Nutr 1996;64:242–8.
16. Gopalan C, Ramasastri BV, Balasubramanian SC. Nutritive value of Indian foods. Hyderabad: National Institute of Nutrition, 1989.
17. West CE, Poorvliet E. The carotenoid content of foods with special reference to developing countries. Alexandria, Va, USA: VITAL, International Science and Technology Institute, 1993.
18. Stoler A. Garden use and household economy in rural Java. Bull Indones Econ Stud 1978;14:85–101.
19. Wasantwisut E, Attig G. Empowering vitamin A foods: a food-based process for Asia and the Pacific region. Bangkok: Institute of Nutrition, Mahidol University, 1995.

Moving from science to public health programs: lessons from vitamin A[1,2]

Alfred Sommer

ABSTRACT The transition from research to the applications of its results is a difficult one. Several stages must be passed through before a discovery can be put to practical use. The development of the current standards for vitamin A is a good example of both a successful and a difficult transition. The importance of the public media and of international organizations, especially the World Health Organization, in any transition is noted. Movement from research to practical applications requires that scientists take an active, sensible leadership role, that mechanisms for disseminating information and for bringing about scientific consensus are used effectively, and that international funding and support agencies take a role. *Am J Clin Nutr* 1998;68(suppl):513S–6S.

KEY WORDS Vitamin A, policy, research, applications, zinc

INTRODUCTION

Moving from research to its mainstream application for the benefit of society can prove as difficult and elusive as making the discovery in the first place. More than a century passed before Edward Jenner's discovery of successful vaccination resulted in the eradication of smallpox; over 40 y after the development of a successful polio vaccine, the disease was finally eradicated from the Western Hemisphere, but not yet from the rest of the world. A physician who noted that children receiving aspirin after tonsillectomies bled more than those not receiving aspirin began prescribing aspirin to prevent myocardial infarction 40 y before we recently rediscovered and adopted this therapy.

Often, the problem lies with the scientist that makes the original discovery. Having created and published the data, he or she commonly moves to a new phase of their research without concern for the relevant application of the knowledge produced. Thus, the persons most deeply involved in the discovery and most passionately convinced of its validity all too often abandon their own work before it has influenced clinical practice or national health policy.

Multiple steps stand between a research discovery and a change in policy. These include, but are not limited to: *1*) making the discovery; *2*) proving, to one's own satisfaction, that the discovery is indeed real; *3*) documenting variability of the results in different populations, environments, and cultures; *4*) overcoming the traditional reticence of other scientists in the field and convincing them of the discovery's validity; *5*) educating policymakers about the discovery and its policy implications; and *6*) devising and establishing programs that actively put the discovery to work.

Several examples epitomize critical stages of success, and others, of failure. One example we'll return to is nutrition in general and vitamin A in particular. EV McCollum, the founding chair of biochemistry at The Johns Hopkins School of Public Health, was a true public health scientist. He not only discovered vitamins A and D (*1*) and revolutionized nutritional science in the process (2), he worked equally hard at teaching homemakers to feed their families more nutritious diets. To spread the word, he published over 100 articles in *McCall's* magazine, served on presidential commissions, and traveled widely, lecturing to home economics teachers. He worked closely with pediatricians, educating them about the relation between rickets (until then a disease of mysterious origin) and the preventive and curative powers of vitamin D. McCollum suffered no loss of scientific standing or credibility for these efforts, a concern repeatedly raised by researchers who fear that promotion of the practical value of their discovery for people's health will detract from their standing as scholars.

VITAMIN A

The development of oral rehydration therapy provides another lesson. Soon after it was clear that a safe oral solution for rehydration of cholera victims was at hand and effective even in remote field settings (3), Alex Langmuir, who had followed its development closely, arrived at the Cholera Research Laboratory in Dhaka on one of his periodic visits. Langmuir asked what the investigators planned to do next. They excitedly discussed a series of studies to explore the physiologic basis of their success, refining and improving the solution. He cut them off. "No," Langmuir declared, "you are going to repeat the practical field demonstration in Bengali adults, Nepalese children, Nepalese adults, Filipino children, etc, and you are going to publish each study in a different journal." They did. His purpose, and the

[1] From The Johns Hopkins School of Public Health, The Johns Hopkins University, Baltimore.

[2] Address reprint requests to A Sommer, The Johns Hopkins School of Public Health, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD. E-mail: asommer@jhsph.edu.

98

result, was to saturate the medical establishment with this new discovery, ensuring that everyone had heard of it (A Langmuir, personal communication, 1987). He helped ensure that they took notice and provided them with evidence that it worked in different cultures and environments and could not be easily dismissed as something that is good for Bengali but not Filipino children.

My own experience with vitamin A deficiency and childhood mortality is consonant with these patterns of response to new discoveries and provides clues about ways to overcome predictable obstacles to practice. Despite McCollum's one-man educational crusade, scientific insights from the first 2 or 3 decades of this century had relatively little effect on the public's health (4). Animal experiments and a smattering of clinical studies strongly suggested that vitamin A deficiency was not only responsible for an important form of pediatric blindness, but for morbidity and mortality as well. Indeed, Stephenson and Clark (5) showed that vitamin A–deficient animals died at excessive rates and suffered severe systematic infections long before their deficiency was severe enough to cause xerophthalmia. Ellison (6) proved, in a classic randomized clinical trial in children hospitalized in London for moderate-to-severe measles, that vitamin A supplementation (as cod liver oil) reduced case fatality rates by 50%. Yet none of this changed health policy, accepted scientific paradigms, or the clinical management of measles.

Many years later, in 1983, we observed that vitamin A status seemed closely linked, in a dose-responsive way, with preschool-age mortality (7). Our publication of these findings was essentially ignored, with the exception of a single inquisitive letter to the editor; no one challenged our observation, publicly agreed with it, or, as far as I know, planned to confirm or refute it. We then launched a randomized trial of periodic large-dose vitamin A supplementation every 6 mo. The conservative intention-to-treat analysis indicated that supplementation reduced preschool-age mortality by >30% (8). The response from those in the nutritional science community was bimodal: some were interested and enthusiastic; most were either nonbelieving or openly hostile. One researcher was quoted in a news article as saying, "If Sommer claimed a 10% impact we might have believed it; but not 30%." We may have convinced ourselves that we were onto something, but we clearly had not convinced the scientific community at large; without at least general agreement among interested scientists, there was little hope of convincing policymakers. Note that the one telling exception was in Indonesia; having been a party to the research, workers there believed and accepted the result and began to address its programmatic implications. Policymakers immediately drew up plans for a nationwide vitamin A intervention program, and included it in their 5-y national plan. Indonesia has continued with an active and productive research program ever since.

Martin Forman, then director of the US Agency for International Development (USAID) Office of Nutrition, instantly recognized the need for replicative randomized trials conducted by different qualified teams in different countries. Only then, he reasoned, would there be a critical mass of evidence from widely scattered populations and environments that would, if valid, capture a group of international and local scientists. After several organizational meetings and half a decade of additional field work, the results (4) and opinions largely coalesced (9; 10; GH Beaton, R Martorell, KA L'Abbe, unpublished observations, 1992). Importantly, policymakers were involved in the research projects in their own countries, even if at an advisory level, thereby setting the stage for their interest and commitment and subsequent action on the results.

As evidence grew, so did international political support. A consensus meeting held at the Rockefeller Study Center in Bellagio, Italy, in 1992 issued a concise series of recommendations and supporting data that was reprinted in mainline medical journals (11) as a means of bringing the debate to closure. The United Nations Activities Coordinating Committee/Subcommittee on Nutrition issued its own analysis. As a result of these published and widely circulated conclusions, scientists and policymakers followed and participated in the worldwide endeavor through involvement in the meetings of the International Vitamin A Consultative Group (IVACG) every 18 mo and actively shared information. Furthermore, the personal commitment of a few highly respected international leaders, including the late James Grant, then director of the United Nations Children's Fund (UNICEF); Hiroshi Nakajima, Director General of the World Health Organization (WHO); and V Ramalingaswami, chair of the International Conference on Nutrition, made control of vitamin A deficiency by the year 2000 an integral goal of the World Summit on the Rights of Children, the International Conference on Nutrition, the World Health Assembly, the Food and Agriculture Organization (FAO), and UNICEF.

Fifteen years ago, few countries were concerned about vitamin A deficiency as a significant public health problem; even fewer were attempting to do anything about it. Although vitamin A deficiency will certainly not be controlled worldwide by the year 2000, more than 60 countries now recognize its importance and are actively engaged in developing or implementing intervention activities.

There is some telling irony to this tale. Soon after proving to our own team's satisfaction that vitamin A prophylaxis could reduce overall preschool mortality by at least 30%, we wondered whether it might not play a major role in the high case-fatality rates associated with measles in African countries, where vitamin A deficiency was not considered a serious or prevalent problem. A small hospital-based randomized study in central Tanzania showed that large-dose vitamin A supplementation resulted in a 50% reduction in the rate of severe measles complications and in measles case fatalities (12). The article was published by the *British Medical Journal* in the section "Research from the South," suggesting to many readers that, whereas the results were too impressive to ignore entirely, the study was at least faintly tainted. Ironically, this was the same journal that had published the identical London study almost half a century earlier (6).

Despite this single study of relatively small size, UNICEF and the WHO officially recommended large-dose vitamin A supplementation for the routine treatment of measles under conditions existing in most developing countries (13). No doubt, the impetus for this response was the growing evidence of the role of vitamin A in preschool mortality as a whole, and particularly in measles-specific mortality. With community-based prophylactic studies now capped by a randomized therapeutic trial, it must have seemed the prudent thing to do even in the absence of replicative data. Subsequent studies proved that that the decision was a wise one (4). With a resurgence of measles and measles deaths in the United States, particularly in children with low vitamin A concentrations, the same recommendation has been adopted by the American Academy of Pediatrics.

An entirely fortuitous event that affected the widespread acceptance of our research shows the importance of the public

media. A colleague, while in flight to a refugee relief camp during the Ethiopian famine of the mid-1980s, was seated next to the then UNICEF director James Grant and told him of the importance of vitamin A supplementation during refeeding to prevent blindness and reduce childhood mortality. On returning to the United States, Grant asked that I brief him and his colleagues on these developments, an event that initiated his and UNICEF'S continuing interest and support for the control of vitamin A deficiency. As research moved forward and the data solidified, UNICEF officers around the world were instructed to inform country counterparts of important developments and to encourage the organization of vitamin A programs. Later, a *Time* magazine article about the Ethiopian famine included a sidebar article about the lifesaving properties of a new "golden bullet," the gelatin capsule used to distribute vitamin A. We have never lived down this sobriquet among our research colleagues, but it served as a powerful marketing tool among policymakers and the public.

Finally, one cannot overestimate the value of WHO publications. Because those at USAID who supported our research and created IVACG recognized early on the importance of having senior leadership of IVACG come from within the UN system in general and WHO in particular, IVACG's first chair, Edward DeMayer, the distinguished WHO nutritionist, arranged to have the recommendations and conclusions of the first major international meeting held in Indonesia in 1974 published in the WHO's *Technical Report Series* (14). These conclusions included the criteria by which vitamin A deficiency could be classified as constituting a public health problem (thereby declaring that vitamin A deficiency could pose an important health problem) and recommended dosages of vitamin A for preventing such a problem and for treating active xerophthalmia. By the second such conference, held 8 y later, the original recommendations could be refined on the basis of data generated during the intervening decade. These, too, were published by the WHO (15), and although the recommendations were never official (which would require the work of expert committees constituted specifically for that purpose), their publication in the *Technical Report Series* provided the appearance of the WHO's imprimatur, which gave the recommendations validity in the eyes of policymakers worldwide that would never have been achieved otherwise.

In summary, moving from science to public health programs requires a group of individuals, including scientists originally involved in the discovery, to take an active, balanced, objective leadership role. It requires a clearly defined series of steps that include ensuring that the conclusions are valid, that the environments in which they are relevant are defined, and that the local scientists and policymakers are involved in the process. Especially important are mechanisms such as IVACG for sharing information and disseminating results (including lessons learned in overcoming programmatic obstacles), bringing the scientific discussion to closure, and widening the network of involved organizations, particularly credible entities like UNICEF and the WHO. Nothing can substitute for a dedicated funding agency (USAID in the case of vitamin A), regardless of the support that others might bring to the table. When the time is right, all appropriate means must be employed to get the word out. Major reports about vitamin A appeared in *AsiaWeek*, *Time*, *The New York Times*, and the mass media around the world: these in turn stimulated other media outlets that drew further political and financial support: in the United States, a television story (on *20/20*, moderated by Hugh Downs, who worked closely with UNICEF) captured the attention of members of Congress who increased their support for USAID's vitamin A and child survival accounts.

CONCLUSIONS

The success of any efforts to translate research into public health programs depends on continuing research that overcomes unanticipated obstacles or simplifies implementation. The smallpox eradication program would have failed in the absence of such research (16). Its original approach, to vaccinate the entire population, proved impractical and was replaced by "surveillance and containment," concentrating resources on vaccinating all those known to have been in contact with an active case. The vaccine, originally heat labile, was lyophilized, thereby simplifying storage and transport. And just as vaccine supplies were running low, along came the simple but brilliantly designed bifurcated needle, which required so little vaccine per application that it effectively quadrupled the world's supply overnight.

Convincing policymakers to implement programs is always difficult. They are constantly dealing, within a politically charged environment, with requests that exceed resources. In the case of vitamin A deficiency, xerophthalmia and blindness were perceived by medical and public health personnel, but by very few policymakers, to have sufficient claim on limited resources. It was only after the relation of vitamin A deficiency to morbidity and mortality became apparent that policymakers became interested. Cost-benefit analyses are useful only to a point: every society interprets these through their own culture (what economists call externalities) in determining what they are and are not most concerned about.

It is clear from the articles in this supplement that our knowledge concerning the role zinc plays in health and disease is rapidly approaching a critical mass. Scientific leaders should set timely targets for follow-up meetings at which additional data can be considered, discussed, and assimilated. Even before then, once consensus is reached on the value of targeted zinc supplementation, meetings should be held with policymakers appropriately experienced with micronutrient issues to consider and compile alternative programmatic strategies. These will begin to stimulate international interest, provoke suggestions and locally appropriate refinements, and serve as a basis for advocacy and commitment.

REFERENCES

1. McCollum EV, Davis M. The necessity of certain lipids in the diet during growth. J Biol Chem 1913;15:167–75.
2. McCollum EV. The newer knowledge of nutrition: the use of food for the preservation of vitality. 1st ed. New York: Macmillan, 1918.
3. Cash RA, Nalin DR, Rochat R, Reller LB, Haque ZA, Rahman AS. A clinical trial of oral therapy in a rural cholera-treatment. Am J Trop Med Hyg 1970;19:653–6.
4. Sommer A, West KP. Vitamin A deficiency: health, survival, and vision. New York: Oxford University Press, 1996.
5. Stephenson M, Clark AB. A contribution to the study of keratomalacia among rats. Biochem J 1920;14:502–21.
6. Ellison JB. Intensive vitamin therapy in measles. Br Med J 1932;2:708–11.
7. Sommer A, Tarwotjo I, Hussaini G, Susanto D. Increased mortality in children with mild vitamin A deficiency. Lancet 1983;2:585–8.
8. Sommer A, Tarwotjo I, Djunaedi E et al. Impact of vitamin A supplementation on childhood mortality: a randomised controlled community trial. Lancet 1986;1:1169–73.

100

9. Fawzi WW, Chameres TC, Herrera MG, Mosteller F. Vitamin A supplementation and child mortality: a meta-analysis. JAMA 1993;269:898–903.
10. Glasziou PP, Mackerras DEM. Vitamin A supplementation in infectious diseases: a meta-analysis. Br Med J 1993;306:366–70.
11. Sommer A. Vitamin A deficiency and childhood mortality. Lancet 1992;339:864 (editorial).
12. Barclay AJG, Foster A, Sommer A. Vitamin A supplements and mortality related to measles: a randomised clinical trial. Br Med J 1987;294:294–6.
13. Expanded Programme on Immunization: programme for the prevention of blindness nutrition. Joint WHO/UNICEF statement on vitamin A for measles. Wkly Epidemiol Rec 1987;62:133–4.
14. Vitamin A deficiency and xerophthalmia. Report of a joint WHO/USAID Meeting. World Health Organ Ser 1976;590:1–88.
15. Control of vitamin A deficiency and xerophthalmia. Report of a Joint WHO/UNICEF/USAID/Helen Keller International IVACG Meeting. World Health Organ Ser 1982;672:1–70.
16. Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Smallpox and Its Eradication. Geneva: World Health Organization, 1988.

PII: S1350-9462(97)00001-3

Xerophthalmia and Vitamin A Status

Alfred Sommer

Epidemiology, Ophthalmology and International Health, Johns Hopkins Medical Institutions, Johns Hopkins School of Hygiene and Public Health, 615 North Wolfe Street, Suite 1041, Baltimore, MD 21205-2179, USA

CONTENTS

Abstract 9
1. Introduction 10
2. Vitamin A 10
3. Vitamin A Status 11
   3.1. Tissue Concentrations 12
   3.2. Functional Tests 14
4. Xerophthalmia 16
   4.1. Nightblindness (XN) 17
   4.2. Conjunctival Xerosis (X1) and Bitot's Spots (X1B) 17
   4.3. Corneal Xerophthalmia 18
      4.3.1. Punctate keratopathy 18
      4.3.2. Corneal xerosis (X2) 19
      4.3.3. Corneal ulceration (X3) 19
   4.4. Retinal Changes (XF) 22
   4.5. Treatment 22
5. Systemic Morbidity and Mortality 24
6. Future Directions 26
   6.1. Research Needed to Reduce the Prevalence and Severity of Deficiency 27
   6.2. Research at the Continuum: Expanding the Known Health Effects of Vitamin A Deficiency 28
   6.3. Research at the Mechanistic Level 28
Acknowledgements 28
References 28

Abstract—The existence of 'fat-soluble A' has been known for over 80 years. But until recently clinicians were almost wholly absorbed by the ocular changes accompanying deficiency (xerophthalmia), and scientists with the vitamin's metabolic role in the rhodopsin cycle.

The past two decades have witnessed a revolution in clinical and scientific concerns. Xerophthalmia is now recognized as a late manifestation of severe deficiency rather than of early, mild deficiency; as the mechanism responsible for half or more of all measles-associated blindness; and as the cause of half a million or more cases of pediatric blindness worldwide. Milder deficiency increases the severity of infectious morbidity, exacerbates iron deficiency anemia, retards growth, and is responsible for one to three million childhood deaths each year.

Scientists are now busy unraveling vitamin A-dependent gene regulation to explain the myriad manifestations accompanying deficiency, while clinicians are designing and supervising programs to improve vitamin A status in over 60 countries, up from only three countries two decades ago. Control of vitamin A deficiency is now a major health challenge and goal of both UNICEF and the World Health Organization (WHO). Reaching that goal requires better parameters for assessing vitamin A status, increased understanding of metabolic pathways responsible for corneal dissolution (keratomalacia) and the molecular and cellular basis by which vitamin A status mediates resistance to infection. These issues are detailed elsewhere (Sommer and West, 1996). © 1997 Elsevier Science Ltd.

Correspondence: Tel.: (410) 955-3540; Fax: (410) 955-0121; e-mail: ASommer@phnet.sph.jhu.edu

Progress in Retinal and Eye Research Vol. 17, No. 1, pp. 9–31, 1998
© 1997 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
1350-9462/98 $17.00 + 0.00

102

1. INTRODUCTION

Xerophthalmia and vitamin A status is particularly topical. Animal experiments, epidemiologic studies and anecdotal reports early in this century suggested the importance of vitamin A for a host of physiologic functions, including growth and survival. Indeed, Bloch, a Danish pediatrician, equated deficiency of the newly-discovered vitamin with poor growth among orphaned children ('dystropia alipogenetica'—abnormalities of growth associated with lack of a lipid-soluble substance) (Bloch, 1921). Stephenson, studying vitamin A-deficient rats, found the vast majority began to die long before they developed ocular abnormalities (Fig. 1) (Stephenson and Clark, 1920). In a controlled therapeutic trial of measles in London, Ellison discovered that vitamin A supplementation (as cod liver oil) reduced case-fatality rates by 50% (Ellison, 1932).

Despite clues to the wide variety of disease processes linked to vitamin A status, scientists and clinicians focused on the dramatic ocular changes (xerophthalmia) to the exclusion of all others. As recently as 1974, an international conference organized by the World Health Organization issued a series of recommendations (based on exceedingly sparse data) for the treatment of xerophthalmia, its classification, and criteria (largely based on the prevalence of various xerophthalmic signs) for determining whether vitamin A deficiency posed a public health problem. An intervention program was judged to have worked only if it reduced the prevalence (or severity) of xerophthalmia (World Health Organization, 1976)

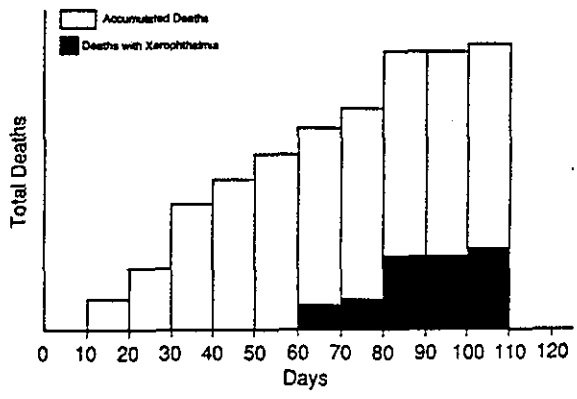


Fig. 1. Cumulative deaths of increasingly vitamin A deficient rats. Many died before and most died without ever developing xerophthalmia. (Stephenson and Clark, 1920.)

Investigations over the past 10 years have dramatically altered our understanding of the nature of vitamin A deficiency and its impact on global morbidity, mortality and blindness. In the process, the international health community has come to recognize that vitamin A deficiency is a major health problem worthy of worldwide, organized attention and intervention. Control of vitamin A deficiency, as much to reduce childhood mortality as to prevent blindness, is enshrined in the Declaration of the Rights of Children (Grant, 1993) and the Plan of Action of the International Conference on Nutrition (International Conference on Nutrition, 1992). Large-dose vitamin A supplementation is recommended by WHO and UNICEF as an essential component of measles therapy (World Health Organization, 1987), a recommendation endorsed by the American Academy of Pediatrics for use in the United States (Committee on Infectious Diseases, 1993).

2. VITAMIN A

Vitamin A is a lipid-soluble substance essential for health. First described by E.V. McCollum in the U.S. (McCollum and Davis, 1913) and Osborne and Mendel (Osborne and Mendel, 1913) in England, it is present as the preformed vitamin (retinol) in meat and dairy products. Animal and fish livers (and their derivatives, like cod liver oil) are particularly rich sources of preformed vitamin A, since it is stored in the liver.

Individuals on vegetable diets, especially those in developing countries, depend to a large degree on provitamin A sources, especially provitamin A beta carotene. While beta carotene is essentially composed of two molecules of retinol, its conversion by the gut to vitamin A is limited by carotene's entrapment within the cellular matrix of vegetables and fruits. By convention, plant sources of beta carotene are considered to provide only one-sixth of their

theoretical vitamin A content (e.g. 'retinol-equivalents'). Recent studies however, suggest the appropriate conversion factor varies by plant species and may be much lower (depee *et al.*, 1995). Availability is only modestly improved by cooking, mashing and other techniques for breaking down vegetable matrices.

Ingested vitamin A, of whatever source, is carried in chylomycra to the liver where it is stored as retinyl ester for future use. Liver stores serve as a 'bank' against seasonal variations in dietary supply, helping maintain a relatively stable, adequate supply to maintain serum retinol levels and the amounts needed by target tissues year round.

Retinol is released from the liver in association with a carrier protein, 'retinol binding protein' (RBP). The combined moiety is known as 'holo RBP'. In situations of severe protein deficiency or other instances resulting in impaired RBP synthesis, release of holo-RBP declines and liver stores of retinol rise. While it is thought that inadequate protein can cause xerophthalmia, there is little evidence that this is the case. The firmest data comes from evidence that xerophthalmic children with low protein stores release relatively modest levels of holo-RBP in response to large-dose (200,000 IU) vitamin A therapy (Sommer *et al.*, 1982b) and suffer premature clinical relapse (Sommer, 1982a).

A wide variety of cellular receptors exist for holo-RBP by which retinol gains entrance to the cell (Ong, 1994). Once inside the cell, vitamin A plays many roles. The elegantly elucidated rhodopsin cycle turns out to be only one. Of more generic and widespread importance is its interaction with nuclear retinoic acid receptors and their impact on gene regulation (Wolf, 1990; Chytil and Riaz-ul-Haq, 1990). Expression of well over 300 genes are already known to be influenced by vitamin A status. No doubt more will be identified, explaining the critical role vitamin A plays in a bewildering array of metabolic processes. The rhodopsin cycle aside, many important clinical expressions of vitamin A status can probably be explained by its impact on cellular differentiation, whether of an immune stem cell or transformation of keratinized into mucous secreting columnar epithelium.

The rapidity of the response to vitamin A supplementation is startling. One hour following a large oral dose of vitamin A supplement, deficient rats develop readily detectable changes in gene products. This would help explain the dramatic impact vitamin A supplementation has on the case-fatality rates and the severity of complications associated with treatment of children already hospitalized with severe, advanced measles.

The recommended daily allowance (RDA) for vitamin A is based upon relatively modest empirical data derived primarily from carefully controlled depletion studies on a small number of otherwise healthy adults (Hume and Krebs, 1949; Sauberlich *et al.*, 1974), extrapolated to satisfy the biologically variable needs of most of the population. The far greater needs of populations living under more trying conditions, especially those with heavy worm loads and frequent diarrhea so characteristic of children in developing countries, are poorly appreciated.

3. VITAMIN A STATUS

To understand the relationship between vitamin A status, xerophthalmia and other bodily processes, it's important to recognize problems inherent in determining 'status' *per se*, and the limitations in interpreting the parameters employed. As deficiency is the subject of this review, we will confine our discussion to recognizing and quantifying degrees of 'inadequate' vitamin A nutriture.

The major parameters for assessing inadequate nutriture can be conveniently divided between those that measure tissue concentrations of vitamin A (e.g. in the serum, liver, breast milk) and those that reflect adequacy of vitamin A-dependent physiologic function (from gene expression at the molecular level to clinically apparent xerophthalmia). Each has greater validity than long-used measures of 'vitamin A intake'. The latter suffers from imprecisions in recall and conversion to imputed retinol equivalents contained in varying diets, as well as yet unidentified

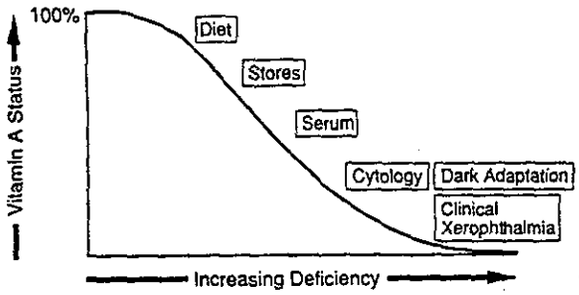


Fig. 2. Schematic relationship between the decline in vitamin A status and the onset of potentially relevant indicators of inadequate vitamin A nutriture. The shape of the curve is illustrative only.

differences in requirements under conditions of varying health and environmental insults.

Dietary 'intake', at its best, measures only intake; it is not a substitute for vitamin A 'status'.

Given what we now know, an inadequate diet (inadequate to 'need') results in gradual depletion of body stores. Circulating levels of serum holo-RBP (e.g. retinol) decline more slowly, since they are homeostatically maintained. As stores and serum levels decline, so does the adequacy of vitamin A delivered to target cells, ultimately manifesting itself in a change in gene products (not yet available as a diagnostic tool) and subsequently clinically manifest abnormalities (Fig. 2). We do not yet possess a single, universally acceptable criterion for '100% adequate' vitamin A status; only alternative approaches, addressing this issue from different perspectives.

3.1. Tissue Concentrations

'Serum vitamin A' has the historical pride of place. It is the most widely measured index of vitamin A status. Individual serum retinol levels are conventionally considered 'normal', 'low', or 'deficient' (Table 1). Several caveats, however, apply:

- Serum retinol is not determined solely by vitamin A status. Inadequate protein synthesis (for whatever reason) (Arroyave *et al.*, 1961) and the response to infectious and non-infectious inflammatory disease (Filteau *et al.*, 1993) can all reduce circulating levels of retinol in the presence of adequate vitamin A stores. Whether they also impair vitamin A-dependent function (e.g. the adequacy of vitamin A at target cells) is not well established.
- Experiments and clinical observations indicate that suggested criteria for 'adequacy' are far from perfect: impaired dark adaptation in otherwise healthy adults is manifest at serum retinol levels between 20 and 30 μg/dl (Hume and Krebs, 1949; Sauberlich *et al.*, 1974), clinically non-xerophthalmic children with serum levels above 20 μg/dl demonstrate impaired functioning, including conjunctival metaplasia (Natadisastra *et al.*, 1988), which are associated with other health consequences (Sommer and West, 1996).
- A significant proportion of children with clinical xerophthalmia have serum levels above 20 μg/dl, while an even larger proportion with seemingly normal eyes have levels below 20 μg/dl (Table 2) (Sommer *et al.*, 1980b).

Table 1. Serum vitamin A: classification of vitamin A 'status'

μg/dl*	μmol/l	Vitamin A 'status'
≥20	≥0.7	Normal
10–20	0.35–0.69	Low
<10	<0.35	Deficient

*Serum levels were traditionally expressed as μg/dl. They are increasingly reported as μmol/l (28.57 μg/dl = 1 μmol/l). In still older literature, 'international units' were used. The reader is cautioned to pay close attention to the unit of measurement.

But none of this should be surprising. Few biochemical tests have cut-offs that sharply delineate 'normal' from 'abnormal'; every criterion will trade off sensitivity for specificity. The distribution of serum retinol levels in a population (or a representative sample) provides a more stable parameter of interest. In this regard, it is evident that serum retinol levels correlate, in a dose–responsive relationship, to the severity of even the mildest manifestations of xerophthalmia (Table 2) (Sommer *et al.*, 1980b; Sommer and Muhilal, 1982; Sommer, 1982a; Sommer and West, 1996).

Liver vitamin A concentration is touted as a more direct measure of 'status' (e.g. stores) than is serum retinol since it is less susceptible to the intervening variables (homeostatic as well as

105

Table 2. Association between xerophthalmia status and serum vitamin A

Clinical status	Distribution of serum retinol levels: Deficient (< 10μg/dl)	Low (10–20 μg/dl)	Adequate (> 20μg/dl)	Total	Mean (μg/dl)	Number of cases
A*						
XN (+), X1B (−)	27%	55%	18%	100%	13.9	174
XN (−), X1B (+)	31%	57%	12%	100%	13.4	51
XN (+), X1B (+)	38%	53%	9%	100%	12.1	79
Neighborhood controls	N/A	N/A	N/A	N/A	17.7	282
Random sample	8%	37%	55%	100%	20.0	268
B†						
X2	62%	34%	4%	100%	8.2	26
X3A (ulcer)	69%	31%	0%	100%	7.2	32
X3A (necrosis)	92%	8%	0%	100%	5.4	25
X3B	80%	20%	0%	100%	5.2	15

*Modified from Sommer *et al.* (1980b)—community-based study.
†Modified from Sommer and Muhilal (1982)—hospital-based study.
XN = nightblindness; X1B = Bitot's spots; controls = neighborhood peers of same age and sex; random sample = peers from throughout the six villages studied; X2 = corneal xerosis; X3A = ulcers and localized keratomalacia; X3B = widespread corneal necrosis (keratomalacia); 'random sample' distribution of serum retinol for XN(−)/X1B(−). Data on XN, X1B, controls and random sample from a 6-village field study outside Bandung, Indonesia. Data on corneal cases were children seen at the Cicendo Eye Hospital, Bandung, Indonesia.

exogenous) affecting release and utilization. Under normal conditions, 90% of vitamin A is stored in the liver, and biopsy specimens as small as 7 mg reportedly provide reasonably precise estimates of liver concentrations (Amadee-Manesme *et al.*, 1984). Olson has developed a rationale for choosing 20 μg/g as the criterion for 'satisfactory' status (Olson, 1987). Despite the potential validity of direct determinations of liver concentration, it is clearly impractical, and under most circumstances dangerous and unethical. An alternative, indirect approach is in use: the relative-dose–response (RDR). Since otherwise adequately nourished but vitamin A-deficient animals accumulate RBP in their liver, they respond to a vitamin A supplement with a large outpouring of holo-RBP. The RDR quantifies the change in serum retinol between baseline, just before the supplement is administered, and 5 hours later. There is no question that this technique can reflect the status of liver stores. In a natural experiment, it was found that children who developed a bout of chickenpox after receiving a large prophylactic dose of vitamin A developed an abnormal RDR more quickly than those who did not (Table 3) (Campos *et al.*, 1987). But a variety of considerations indicate the shortcomings and variability of this response (Sommer and West, 1996). Essentially all the correlations available from the RDR test are provided by the simpler, single sample serum retinol determination. If RDR has any value, it is in specially controlled research undertakings.

Table 3. Effect of varicella infection on vitamin A status

Days after supplementation	Number of infected children*: +	−	Retinol (μg/dl infection: +	−	RDR positive infection: +	−
0	57	36	37 ± 14	38 ± 14	42%	38%
30	42	30	49 ± 9	47 ± 9	0%	0%
120	31	31	38 ± 7	41 ± 6	10%	0%
180	29	31	26 ± 9†	41 ± 14	74%	10%

*Varicella infection at approximately 3 months.
†$P < 0.001$ for difference between infected and control groups.
(From Campos *et al.*, 1987)

3.2. Functional Tests

Functional tests assess the physiologic adequacy of vitamin A status. By definition, 'adequacy' will vary with the physiologic response being examined and the sensitivity of the test procedure to detect an abnormality.

One of the newest approaches, a scotopic vision tester, provides a field-practical, simplified approach to dark adaptometry suitable for evaluating young children (Sanchez *et al.*, 1997; Congdon *et al.*, 1995). One can objectively record the light intensity at which a consensual pupillary response occurs (in the youngest children) or a subjective response (when they first claim to see the light) in an older child (3 years and above). Preliminary results in Americans, Indonesian and Indian children are encouraging. The pupillary threshold score in Indian children is directly related to serum retinol (Fig. 3) while treatment with vitamin A resulted in a dramatic improvement in the response of Indonesian children (Fig. 4).

An alternative functional approach, already in wide use, applies a millipore filter to the inferonasal conjunctiva (conjunctival impression cytology—CIC:A). When peeled off, over 90% carry with them a superficial layer of conjunctival epithelium which can then be fixed, and at one's leisure, stained and studied for keratinizing metaplasia. Early results in a carefully controlled animal model (Hatchell and Sommer, 1984) have been born out in a series of human studies

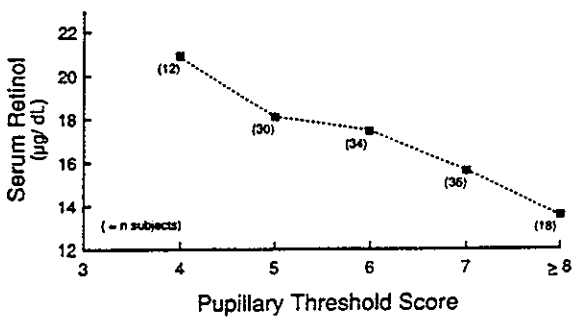


Fig. 3. Comparison of serum retinol level and pupillary threshold. The lower the serum retinol, the higher the pupillary score (the brighter the light needed to elicit a response). (From Sanchez *et al.*, 1997.)

(Wittpenn *et al.*, 1986). In a carefully studied group of Indonesian children, there was a direct correlation between the prevalence of abnormal cytology and other evidence of vitamin A deficiency; the more severe the other evidence, the greater the likelihood of deficiency (Table 4) (Natadisastra *et al.*, 1988). Over 90% of children most likely deficient were positive, versus 6% of those least likely to be deficient. While a large number of reports indicate the potential validity of this approach under widely varying cultures, environments and conditions, problems have arisen in standardizing readers, particularly when the sampled tissue has 'borderline' characteristics.

The potential value of these physiologic parameters is the ease with which they can be carried out. Serum retinol suffers from the problems associated with obtaining blood samples (often a cultural, and increasingly a health concern), and the need for carefully preparing, transporting and storing the volatile specimens and the need for expensive equipment and a skilled staff to run the determinations.

The appearance (and prevalence) of clinical xerophthalmia represents the grossest functional indicator of status, but its relative infrequency requires very large sample sizes, something obviated by using tests of milder, earlier dysfunction. It also suffers from validity problems (lack of an objective measure of clinically-claimed night-blindness and the persistence of some Bitot's spots—see Section 4).

We await the ideal test: an early measure of genetic disregulation secondary to mild vitamin A deficiency. In the meanwhile, we need to content ourselves with perfecting and simplifying measures of serum retinol and assessments of functional status like CIC and scotopic vision. Several investigators have shown that children with 'deficient serum retinol' do not have abnormal CIC and vice-versa. While these tests, in these investigators hands, could and have been used to impugn the validity of CIC, they could just as easily impugn the validity of the serum retinol determinations. In all likelihood, these parameters measure different manifestations of the same underlying problem. Interestingly, in most studies where individuals

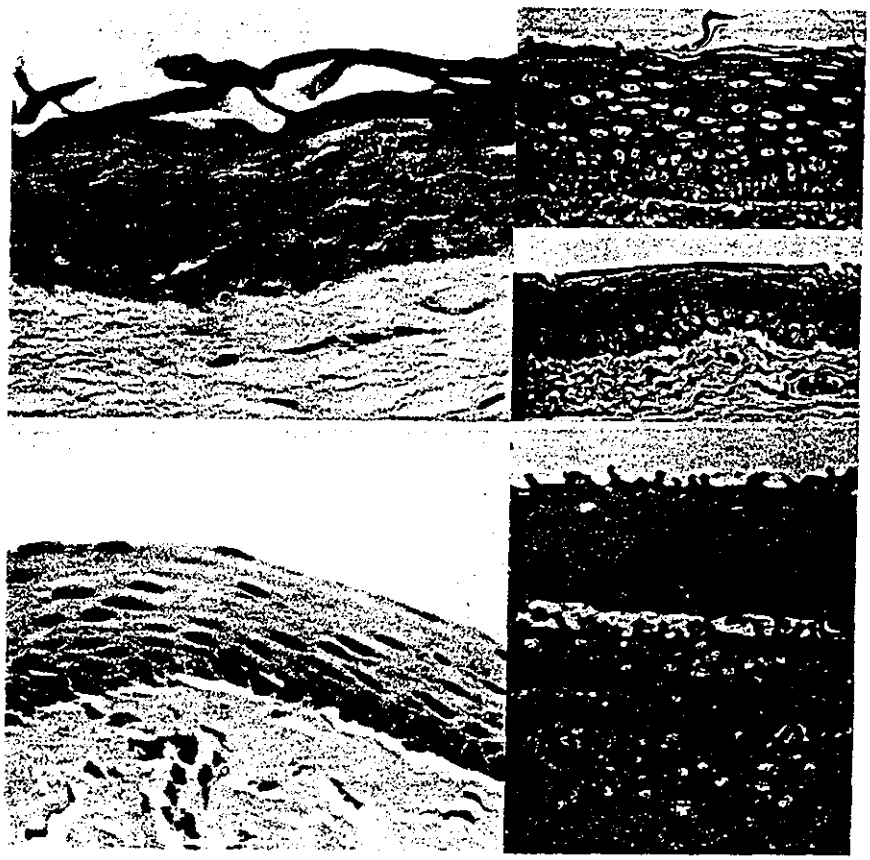


Fig. 4. Top left: By light microscopy, pretreatment temporal conjunctival biopsy (right eye) reveals a prominently keratinized surface (Dane's, ×528). Top right: Phase-contrast microscopy of same area resolves surface keratinization, kerathohyaline granules, acanthosis, and dyskeratosis (paraphenylenediamine, ×480). Bottom left: Biopsy of temporal conjunctiva (left eye) seven days following systemic vitamin A appears normal by light microscopy (hematoxylin–eosin, ×528). Middle right: Phase contrast photomicrograph of same area shows decrease in epithelial thickness (cf. top right; paraphenylenediamine, ×480). Bottom right: TEM (transmission electron microscopy) of same area illustrates partial return of surface membrane infoldings and microvillae (×10,700). (From Sommer *et al.*, 1981a Sommer, 1982a.)

have seemingly incompatible tests, the prevalence of abnormal results in the population at large is similar, regardless of which test is employed.

The major interest in developing a valid, objective measure of vitamin A status is for research purposes, where one needs authoritative validation of the vitamin A-related parameter of interest, and for population assessment to identify communities in need of intervention and as a means of gauging its success. These are now rarely needed for diagnostic purposes, particularly in third world countries. The tests, particularly biochemical assays, are far more expensive than treatment, which is both cheap and harmless. Furthermore, testing can delay urgently needed therapy. Individual diagnostic testing has a place, primarily in developed countries among individuals not expected to be deficient, for the management of patients at risk of deficiency from malabsorbtive states (e.g. cystic fibrosis) or on bizarre diets, or of anyone with classical signs and symptoms and potentially predisposing conditions (cirrhosis, enteritis, alcoholism). Once the test is performed or blood drawn, suspicious corneal ulcers should be treated as if they might well be due to vitamin A deficiency.

Table 4. Prevalence of abnormal CIC versus vitamin A 'status'

Vitamin A status	Patient characteristics	Prevalence of abnormal CIC: Number	Abnormal (%)
1. Definite deficiency	• XN(+) plus X1B(+) responding to vitamin A • Serum retinol <20 μg/dl	14	93
2. Probable deficiency	• Bilateral X1B(+) responding to vitamin A	22	82
3. Probable deficiency	• XN(+) responding to vitamin A • serum retinol <20 μg/dl	15	67
4. Possible deficiency	• Unilateral X1B(+) [XN(−)] responding to vitamin A	8	50
5. Possible deficiency	• Normal exam • Serum retinol <20 μg/dl	26	46
6. Borderline deficiency	• XN(+) plus retinol ≥20 μg/dl or normal exam and retinol 20–25 μg/dl	43	14
7. 'Normal'	• Normal exam • Serum retinol >25 μg/dl*	18	6

*Level > 30 μg/dl would have been preferred, but there were too few such subjects
(From Natadisastra *et al.*, 1988)

4. XEROPHTHALMIA

Despite its Greek origin (xerosis = dry; ophthalmia = inflamed eye), xerophthalmia includes all ocular manifestations of vitamin A deficiency (nightblindness to keratomalacia) classified by international agreement (Table 5). All the milder changes, from nightblindness (XN) to corneal xerosis (X2), are entirely reversible without persistent sequelae (for a minor exception, see 'Bitot's spots'). Any degree of corneal ulceration will leave a scar of varying extent and density. The severest form of corneal melting (X3B) often presents with limbus to limbus necrosis. Such eyes cannot be salvaged, resulting in staphylomas and/or phthisis.

Detailed color plates of each of these manifestations, and their response to therapy, are available elsewhere (Sommer, 1982a; Sommer and West, 1996; Sommer, 1995).

The relationship between the 'severity' of xerophthalmia and the severity of vitamin A deficiency is evident from the correlation between clinical presentation (most severe manifestation) and serum retinol (Table 2). The more severe the manifestation, the lower the mean retinol and greater the proportion of cases with 'deficient' (<10 μg/dl) levels (Sommer *et al.*, 1980b; Sommer and Muhilal, 1982).

Table 5. Clinical classification of xerophthalmia (WHO, revised 1981)

Night blindness (XN)
Conjunctival xerosis (X1A)
Bitot's spot (X1B)
Corneal xerosis (X2)
Corneal ulceration/keratomalacia <1/3 corneal surface (X3A)
Corneal ulceration/keratomalacia ≥1/3 corneal surface (X3B)
Corneal scar (XS)
Xerophthalmia fundus (XF)

However, this correlation does not, as had long been thought, represent an inevitable continuum of progressive severity. Some individuals with the severest manifestations, particularly varying degrees of corneal melting (X3), may never have manifested either nightblindness (XN) or Bitot's spots (X1B). While other issues may play a role, most cases are probably explained by acute decompensation of what had previously been marginal deficiency. While a number of events can cause sudden and severe deterioration in vitamin A status, the most common is measles. Measles is regarded as 'responsible' for 80% of pediatric blindness in Africa (Foster and Sommer, 1986; Chirambo and BenEzra, 1976; Schwab and Kagame, 1993) and for at least 50% in Indonesia (Sommer, 1982a). Measles can markedly depress circulating levels of serum retinol (Reddy *et al.*, 1986a; Laditan and Fafunso, 1981) which often rises spontaneously once the acute phase has passed. The cause of the acute deterioration may be multifactorial: a disturbance in release and transport as well as increased metabolic demand. Regardless, a child with severe measles will not report (and will be difficult to test for) nightblind-

ness, while conjunctival and corneal xerosis require days, if not weeks of keratinizing metaplasia to make themselves known. The corneal stroma, on the other hand, seems exquisitely sensitive and rapidly responsive to severe deficiency, and so can present earlier.

A second phenomenon also contributes to the apparent confusion: conjunctival xerosis is masked or (more likely) reversed by conjunctival inflammation (Sommer, 1982b), a near universal accompaniment of measles even in the absence of corneal destruction.

In a carefully conducted correlation of clinical and biochemical parameters and response to treatment, half the cases of measled-associated corneal ulceration presenting to a mission hospital in central Tanzania were secondary to vitamin A deficiency (Foster and Sommer, 1987). Approximately one-fifth were due to herpes, an important cause of corneal ulcers, particularly in measles (Sanford-Smith and Whittle, 1979), though in Tanzania they were more likely than xerophthalmic ulcers to be mild and unilateral.

It is estimated that xerophthalmia (of varying severity) afflicts five to ten million children and blinds another 500,000 to one million every year.

4.1. Nightblindness (XN)

Nightblindness itself is not of particular concern. It is useful, however, in identifying individuals likely to be vitamin A deficient and therefore at greater risk of more severe xerophthalmia and other complications of deficiency (e.g. infectious morbidity and mortality—see Section 5). Since vitamin A deficiency tends to cluster in families and communities (Sommer, 1982a; Katz *et al.*, 1993; Sommer *et al.*, 1980b), it identifies a population in need of attention. Finally, nightblindness is often the most prevalent form of xerophthalmia and therefore useful for classifying populations in whom vitamin A deficiency constitutes a public health problem (World Health Organization, 1982). Because it is readily reversed in preschool age children, it is a useful index of the impact of intervention programs.

In addition to preschool children, pregnant women often become nightblind, usually in their third trimester. The condition generally disappears spontaneously 2–4 weeks following delivery, presumably from improved diet. A recent study in Nepal, however, indicated that third trimester nightblindness did not always respond to vitamin A therapy, suggesting potential involvement of coexisting zinc deficiency (K. West, personal communication, 14 October 1996).

The new scotopic testing apparatus (see Section 3.2) is not as sensitive as full-scale dark adaptation, but much simpler and more practical for field use, particularly in young children. However where vitamin A deficiency is both endemic and prevalent, a local term for the condition is usually highly sensitive and specific (Sommer *et al.*, 1980b), an observation that has been amply confirmed in many cultures (Stoll *et al.*, 1985). The trick is finding the appropriate local term, which often translates as 'chicken blindness' (chickens lack rods and therefore cannot see under low levels of illumination).

Nightblindness is usually not recognized until the child begins to walk about or feed itself, and is exacerbated by photic stress (such as flying a kite on a sunny day—the only evening the parents may note the condition).

Nightblindness secondary to vitamin A deficiency usually responds within days to high dose therapy (Dowling and Wald, 1960; Sommer *et al.*, 1978; Sauberlich *et al.*, 1974).

4.2. Conjunctival xerosis (X1) and Bitot's Spots (X1B)

Conjunctival xerosis, dryish, sandy-like changes of the conjunctiva, are the clinical reflection of underlying keratinizing metaplasia. Taken to their extreme, the conjunctiva looks like skin, a degree of severity rarely encountered because corneal changes or death tend to intervene. The truly abnormal surface almost always contains some degree of white, foamy, cheesy material that can be readily wiped away. When localized, this overlying material and the xerotic bed below are known as a Bitot's spot, which is practically pathogonomic for present (in very young children) or past vitamin A deficiency.

The characteristic conjunctival abnormalities of xerophthalmia generally begin (and remain most

severe) in the temporal limbus; with persistent deficiency, presumably of increased severity, the nasal, inferior and superior quadrants become sequentially involved (Sommer, 1982b; Semba *et al.*, 1990). The histopathologic changes are generally more widespread than the clinical appearance might suggest: among patients with active vitamin A deficiency in whom Bitot's spots appear limited to the temporal quadrant, biopsies of the inferonasal quadrant show similar, if not as prominent, abnormalities (Sommer *et al.*, 1978); Sommer *et al.*, 1981a). These include loss of goblet cells, an irregular maturational sequence and disorganization of the basal layers (Fig. 4). The surface layers are commonly keratinized, while the heaped-up whitish material covering the Bitot's spot contains a mix of frayed keratin and gram-positive bacilli (the so-called 'Xerosis bacillus', a diphtheroid) (Sommer *et al.*, 1981a; Reddy, 1965; Damien-Gillet, 1958).

While new Bitot's spots in young preschool-age children generally shrink and disappear within 1–4 weeks of systemic vitamin A therapy, this is not invariable. Failure to respond has given rise to considerable confusion in the literature. What generally seems to occur, is that chronic or long-standing deficiency results in localized dysplasia, usually limited to the temporal quadrant. Children with non-responsive lesions are older, have higher baseline vitamin A levels, lesions more likely to be limited to the temporal quadrant (suggesting environmental exposure may play a role), and less likely to be accompanied by other evidence of active xerophthalmia (nightblindness, punctate keratopathy) (Sommer *et al.*, 1980a). While biopsied lesions exhibit changes typical of conjunctival xerosis and Bitot's spots, these are sharply demarcated from the surrounding conjunctiva, which is entirely normal in appearance (Sommer *et al.*, 1981a). Sinha made an extremely important observation among a cohort of preschool children he followed in West Bengal, half of whom received large-dose vitamin A prophylaxis every 4 months and the other half placebo. None of the vitamin A recipients who had not had Bitot's spots at baseline, but a substantial proportion of placebo recipients, developed Bitot's spots during the traditional season of vitamin A deficiency. Among those who had had Bitot's spots previously, however, the spots waxed and waned (sometimes disappearing entirely until the next deficiency season, when they reappeared), regardless of whether they had received vitamin A prophylaxis or not (Sinha and Bang, 1976)

As expected of a localized area of dysplasia, once excised, non-responsive Bitot's spots do not return unless vitamin A status declines.

These observations strongly suggest Bitot's spot prevalence would be a relatively insensitive indicator of the impact of a vitamin A control program. Only incidence, the rate of development of Bitot's spots in 'virgin' conjunctiva, is a reliable index suitable for assessing the response of vitamin A status to an intervention program, even among preschool-age children. Assessment of a large-scale vitamin A supplementation program in Indonesia bears this out: the number of new cases of Bitot's spots among vitamin A recipients was only one-fifth that of the others, while the prevalence of all Bitot's spots remained similar in the two groups (Djunaedi *et al.*, 1988).

4.3. Corneal xerophthalmia

4.3.1. *Punctate keratopathy*

The earliest clinically detectable change in corneal appearance is punctate keratopathy, densest in the inferonasal quadrants (Sommer *et al.*, 1979). These are readily detected as fluorescein positive spots by slit lamp examination. They are not part of the xerophthalmia classification because they had not been noted when the classification was originally devised. They are already prevalent among cases of nightblindness (60%) and Bitot's spots (75%) (Sommer *et al.*, 1980a), and do not appear to represent a direct threat to vision or ocular integrity. They disappear rapidly and entirely with vitamin A supplementation. Similar lesions have been reported in biochemically deficient, asymptomatic adults (Newman *et al.*, 1994). In early deficiency (xerophthalmia, Bitot's spots), the cornea appears grossly clear. With more severe deficiency, the punctate keratopathy becomes denser and spreads superiorly. When sufficiently advanced, the cornea may develop a mild, superficial haze apparent by

hand light examination, justifying the diagnosis of corneal xerosis (X2).

The origin of the punctate lesions is unclear. By the time they cover the cornea, they no doubt represent metaplasia of the corneal epithelium. Earlier, milder lesions may reflect mild, localized metaplasia exacerbated by changes in the environment that bathes the corneal surface; a 'toxic effect' (as yet unidentified) secondary to material comprising the Bitot's spots, particularly their saprophytic bacilli; a non-specific response to reduced tear production demonstrable among patients with xerophthalmia (Sommer and Emran, 1982; Sommer, 1982a); and/or a reduction in the mucous layer of the tear film secondary to widespread disappearance of bulbar conjunctival goblet cells accompanying the early metaplasia associated with conjunctival xerosis (X1) (Sommer *et al.*, 1980a; Sommer *et al.*, 1981a). Goblet cell density is a sensitive indicator and potentially a contributing determinant of ocular surface disorders (Kinoshita *et al.*, 1983; Rao *et al.*, 1987).

The time course for resolution of punctate keratopathy following vitamin A therapy parallels the return of goblet cells (Sommer and Green, 1982). Goblet cells are not, however, the only source of glycoproteins in the tear film, and goblet cell counts can be depressed out of all proportion to modest reductions in tear film mucin (Kinoshita *et al.*, 1983).

4.3.2. *Corneal xerosis (X2)*

Corneal xerosis is a clinical diagnosis based upon a hazy appearance of the corneal surface on handlight examination. By slit lamp, mild xerosis consists of extensive staining and non-staining punctate lesions. With more extensive xerosis, a thickened, hazy, keratinized surface, usually densest inferiorly, is apparent. This keratinized layer may detach in parts, scrolling away, leaving a clear corneal surface, or superficial ulcer, below. Microcysts are sometimes present, becoming typical punctate staining lesions within days of supplemental vitamin A therapy. Slit lamp examination may also reveal stromal edema, often marked, in the presence of an otherwise intact, non-inflamed cornea.

In severe cases, the corneal surface develops a dry, 'non-wettable' 'peau d'orange' appearance. In rare instances, Bitot's spot material and thick xerotic plaques accumulate on the corneal surface, usually centrally.

Following vitamin A therapy, the cornified layer, if present, peels off, leaving an intact, clear surface below. The last area to slough is generally the interpalpebral zone, presumably because it is subject to the least lid abrasion. In contrast, the punctate lesions either disappear or form dense collections of non-staining specks (which may persist indefinitely) inferiorly.

The shedding of xerotic plaques or thick keratinized layer may take with it underlying basal epithelium and stroma, initiating an ulcerative process (Sommer, 1982a).

4.3.3. *Corneal ulceration (X3)*

Xerophthalmic ulceration (X3) ranges from small, characteristically sharp-margined ulcers generally located in the periphery of the cornea, to full thickness, near limbus-to-limbus dissolution. Despite this diversity, each is, to a large degree, seemingly typical of the disease, if not unique, in presentation and in pathogenesis (Sommer, 1982a; Sommer and West, 1996).

Isolated ulcers, presumably the mildest stage, are generally 1–2 mm in diameter and most often located in the inferior quadrant. Occasionally in carefully studied patients, these appear to arise from the rupture of a subepithelial bullae that accompanies corneal xerosis, or in areas denuded by the traumatic separation of keratinized corneal epithelium. In their early stage, they are most often only partial thickness in depth, sharp-margined, and with little evidence of infiltration. When they do perforate, peripheral iris most often plugs the wound, maintaining an intact anterior chamber.

Despite a common belief that xerophthalmia occurs in 'white and quiet' eyes, the vast majority are, in fact, injected, 20% accompanied by a hypopion.

More severe involvement generally presents as larger (or rarely, multiple) ulcers. Even more unique is the occurrence of a large bulging mass involving the entire lower one-third or more of the

cornea. Brown-greyish in appearance, it typically has a glistening surface.

All of the lesions described above respond rapidly to systemic vitamin A. The small peripheral ulcers appear to granulate inward, resulting in a scar of varying density. Where the iris has become incorporated into the wound, as is common, a small, dense adherent leukoma remains. As will be seen, it is not at all clear that adherent leukomas invariably represent sequelae of perforated ulcers. What little histopathologic material of isolated ulceration exists suggests that stromal necrosis can occur posterior to an intact epithelium, the iris and cornea adhering secondarily to corneal swelling (that narrows the anterior chamber) and anterior chamber inflammation (Sommer, 1982a). The mechanism of corneal necrosis remains obscure, but in one well-studied case the edges were sharp-margined, bacteria were absent, there was a paucity of inflammatory cells and the keratinized corneal epithelium was intact (Figs 5 and 6) (Sommer *et al.*, 1982a). Smith also commented on the paucity of inflammatory cells in a case of keratomalacia in an emaciated American female consuming a limited diet (Smith *et al.*, 1975).

Larger, denser areas of 'liquefactive necrosis' also respond to vitamin A. Over 2–4 weeks, the bulging mass flattens as if by deturgescence, and is replaced by a dense scar, usually occupying a surprisingly small area, preserving the visual axis (Sommer, 1982a; Sommer and West, 1996).

Classic keratomalacia, presumably an extention of localized necrosis described above, presents as total dissolution involving all of the cornea. Vitamin A treatment has virtually no effect, other than speeding the slow process of scarring built upon a scaffolding of thin stroma and/or adjacent iris. The eye is inevitably lost, though prompt treatment may preserve the other eye and the child's life. This severest of ocular changes is generally associated with low, often non-detectable levels of serum retinol, severe protein-energy malnutrition, Kwashiorkor, respiratory disease and diarrhea—a severely ill, often moribund state. Under less than ideal conditions, case-fatality rates probably approach 90% (Sommer *et al.*, 1981b; Sommer, 1982a).

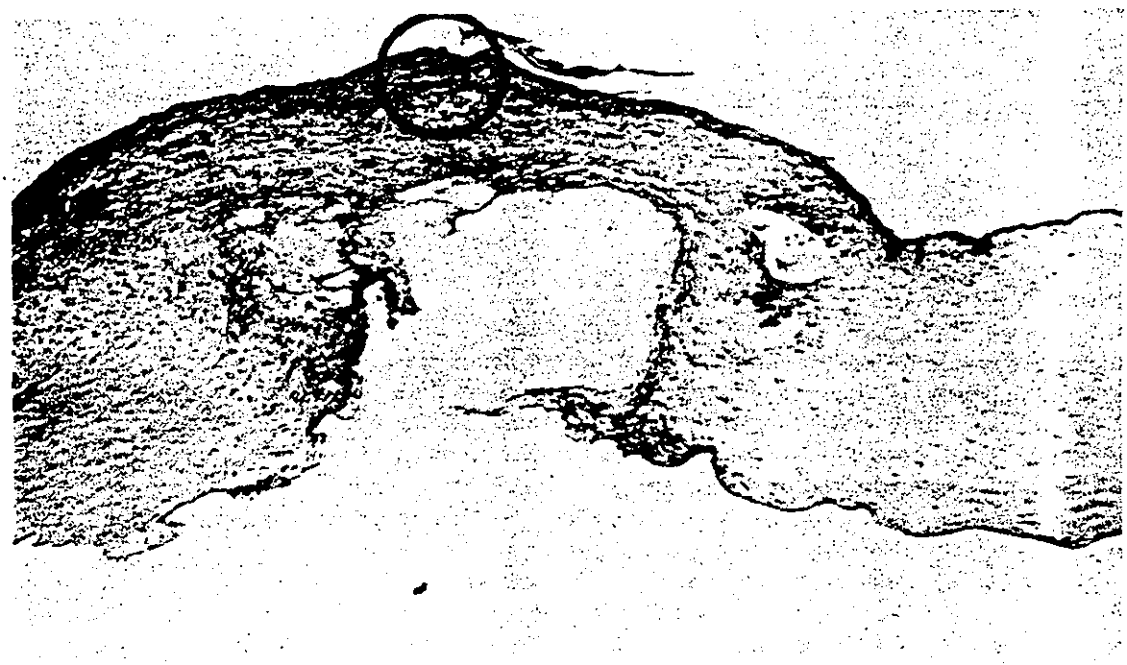


Fig. 5. Xerophthalmic corneal ulceration (X3A) showing central area of lesion. Most of the stroma is destroyed as is Descemet's membrane behind. The sharply delineated saucer-shaped configuration of involvement is readily apparent. The entire area is covered by keratinized epithelium (hematoxylin-eosin, ×30). (From Sommer *et al.*, 1982a; Sommer, 1982a.)

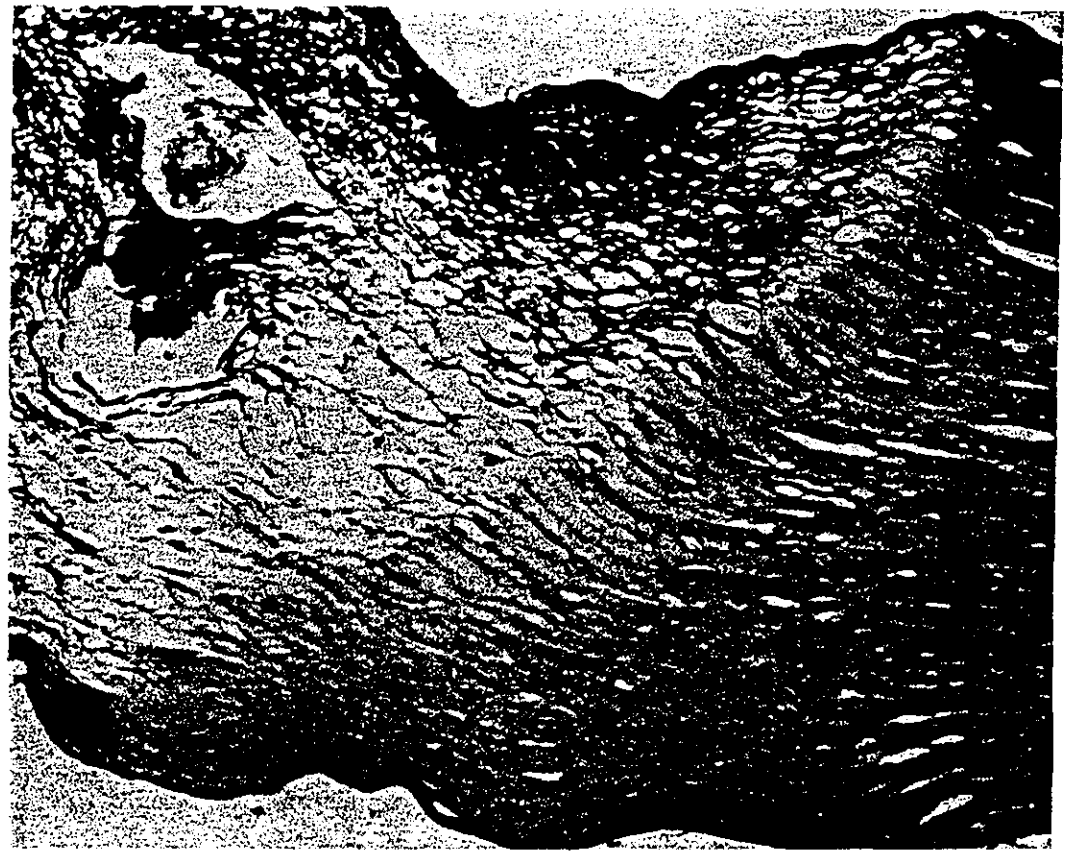


Fig. 6. Corneal ulceration (X3A). The sharply focal nature of the lesion, and lack of both bacteria and significant inflammatory infiltrate, are readily apparent (hematoxylin–eosin, × 100). (From Sommer *et al.*, 1982a; Sommer, 1982a.)

Traditional mechanisms evoked to explain corneal ulceration seem not to apply. Dissolution in the presence of an intact epithelium, the paucity of inflammatory cells and general absence of microbial invasion suggest alternative mechanisms must, in most instances, be at work. While some histopathologic cases demonstrate bacterial and fungal invasion and abscesses, these usually represent late stage disease. There is, of course, no reason why infection could not participate in the process in some cases, or result in secondary invasion in the rest, but it does not appear to be required for initiating xerophthalmic ulceration and necrosis.

Infection is commonly proposed as an important contributory factor. Animal models suggest corneal xerosis increases susceptibility to topically applied pseudomonas (DeCarlo *et al.*, 1981), and pseudomonas and other pathogenic organisms are sometimes recovered from xerophthalmic eyes of malnourished children (Valenton and Tan, 1975). But this is very different from proof that it causes the disease.

In a carefully controlled bacteriological study in Indonesia, the proportion of eyes from which pathogenic bacteria could be recovered was twice as great among cases of X2 than among controls, confirming that the keratinized surface is a hospitable environment for colonization. The rate of microbial recovery was no greater from ulcerated than from non-ulcerated (X2) eyes, however. In a comparison evaluation, one eye of every child admitted with typical xerophthalmic ulcers bilaterally was randomized to receive intensive topical antibiotic therapy (in addition to systemic vitamin A) in one of the two eyes. The two eyes healed equivalently in all cases (Sommer, 1982a).

Finally, if infection plays any role other than end-stage invasion and superficial early colonization, it cannot be a decisive one. Xerophthalmic corneal ulcers heal rapidly in response to systemic vitamin A (Pillat, 1929; Sommer *et al.*, 1980d) or topical retinoic acid (Sommer, 1978).

Despite the growing literature on corneal changes in vitamin A-deprived animals (Sommer and West, 1996), including increased susceptibility to stromal melting following trauma (Sendele *et al.*, 1982; Twining *et al.*, 1985; Seng *et al.*, 1980), we still lack a clear understanding of the pathogenesis of xerophthalmic ulceration and keratomalacia. Vitamin A receptors have been identified at all layers of the normal cornea, and must be there for a purpose (Wiggert *et al.*, 1977), while vitamin A seems to be needed for normal glycoprotein synthesis (Kim and Wolf, 1974). More work along these lines are needed.

4.4. Retinal Changes (XF)

Retinal changes observed in older, presumably chronically recurrent cases of vitamin A deficiency are largely a curiosity. They appear as fine, white dots or mottling that eventually fades with treatment (Mikamo, 1915; Uyemura, 1928). They can also recur with considerable speed (Sommer *et al.*, 1978). The dots appear small and discrete, deep to the vessels and distributed about the equatorial regions of both eyes (Sommer *et al.*, 1978; Teng-Khyoen-Hing, 1959; Bors and Fells, 1971). They tend to remain outside the vascular arcade. The more chronic the deficiency, the larger the number of lesions (Fells and Bors, 1969) which appear to represent mottled depigmentation of the pigment epithelium (Sommer *et al.*, 1978). Functionally, their distribution coincides with reduced retinal sensitivity, which responds more rapidly to improved vitamin A status than does the retinal appearance (Sommer *et al.*, 1978).

The origin of the lesions remains obscure, but severe vitamin A deficiency in animals disrupts the outer segments of rods, followed by degeneration of both rods and cones (Carter-Dawson *et al.*, 1979) and loss of pigment epithelium (Dowling and Wald, 1958).

4.5. Treatment

Xerophthalmia is a medical emergency. Vitamin A is vital to saving the eye as well as the child (see Section 5).

As already noted (Sections 4.1—4.3), all degrees of xerophthalmia respond rapidly to systemic vitamin A except eyes with limbus to limbus keratomalacia. Bloch, who first recognized the clinical connection between xerophthalmia and deficiency of the newly described 'fat soluble A' believed that intramuscular injection of (vitamin A-rich) cod liver oil speeded healing (Bloch, 1924). While injections of oily preparations of vitamin A probably provide some degree of benefit (Chu and Lin, 1938; Perreira *et al.*, 1967), relatively little vitamin A is released from the injection site (Table 6) (Sommer, 1982a; Srikantia and Reddy, 1970). Unfortunately the world is overrun with injectable oily preparations which remain in wide use because physicians like to give injections (and patients like to receive them), and local manufacturers find it easier to produce vitamin A esters in oil. Recognizing that oily preparations work poorly by injection, but fearful that any preparation administered orally would work more

Table 6. Serum vitamin A (μg/dl) among single-dose vitamin A recipients

Time since initial dose*		Treatment regimen†		
		Oral	IM (water)	IM (oil)
0 hours	$\bar{x}$	8.0	9.7	5.8
	S.D.	4.7	5.1	4.2
	n	10	11	12
4 hours	$\bar{x}$	41.4	70.0	6.0
	S.D.	32.6	44.1	4.5
	n	7	11	12
1 day	$\bar{x}$	28.0	108.7	7.6
	S.D.	13.6	47.3	10.0
	n	9	11	9
3 days	$\bar{x}$	30.5	83.5	9.4
	S.D.	33.5	46.7	6.1
	n	6	11	10
7 days	$\bar{x}$	24.4	28.0	15.9
	S.D.	10.3	6.7	13.4
	n	5	5	7

*Limited to cases of corneal involvement included in the analysis of clinical response.
†Oral: 200,000 IU oil-miscible vitamin A PO; IM (water): 100,000 IU water-miscible vitamin A intramuscularly; IM (oil): 200,000 IU oil-miscible vitamin A intramuscularly.
(From Sommer, 1982a)

115

slowly than a parenteral dose, official recommendations from 1972 (World Health Organization, 1976) stressed the use of water-miscible intramuscular vitamin A. Subsequent, randomized clinical trials in children with xerophthalmic ulceration demonstrated that oral large-dose vitamin A was equivalent, in its biochemical and clinical impact to parenteral treatment (Sommer *et al.*, 1980d). This is now the recommendation of choice (World Health Organization, 1982; WHO/UNICEF/IVACG Task Force, 1997). The practical implications far outweigh the scientific: oral high-dose vitamin A capsules are cheap, widely available, safely and (immediately) available for use by trained non-medical personnel, obviating the cost, risks and delays inherent in requiring needles and syringes. Recommended doses (Table 7) are given immediately, again the following day (to ensure an adequate, urgent amount was received and to begin 'topping up' liver stores), and again 2–4 weeks later (further increasing stores). The value of three, as opposed to a single dose has never been documented under routine conditions. But the relatively low and poorly maintained holo-RBP response and rapid clinical relapse among protein deficient children (Large *et al.*, 1980; Sommer *et al.*, 1982b; Sommer, 1982a) suggests the value of repeated dosing.

It has been argued that diarrhea, with its attendant malabsorption, might interfere with the effectiveness of a large oral dose of vitamin A (Nalin *et al.*, 1980). In fact, studies with labeled vitamin A indicate frequent diarrhea reduces retention of a large oral dose from 87% to 70% (Reddy *et al.*, 1986b). Not surprisingly, this had no effect on the clinical response to oral therapy (Sommer, 1982a; Sommer *et al.*, 1980d; Sommer, 1980). Since the liver of a 3-year-old, 7.5 kg child contains approximately 30,000 IU vitamin A, 70% retention of even a 100,000 IU dose would suffice to replenish liver stores, assuming normal liver function and adequate protein synthesis.

Vitamin A is present in tears of humans and animals (Ubels and MacRae, 1984; Speek *et al.*, 1986). The concentration is affected by systemic vitamin A status (Van Agtmaal *et al.*, 1988). Topical vitamin A has therefore been suggested for use as adjunctive treatment. However, topical vitamin A cannot be substituted for systemic vitamin A, which is required not only to restore ocular needs but those of other tissues throughout the body, from the bronchopulmonary lining to immune competent cells. Topical retinoic acid can speed reversal of corneal keratinization in deficient rabbits (Van Horn *et al.*, 1981). In conjunction with systemic (oral) vitamin A, topical retinoic acid also speeds healing of corneal ulcers in children, however not to a clinically significant degree, and occasionally results in denser scarring (Sommer and Emran, 1978; Sommer, 1983). It's probably wise to restrict vitamin A therapy to large, oral doses.

A variety of adjunctive treatments are in order (Sommer, 1995): reversing protein deficiency, thereby increasing protein synthesis and the availability of RBP; managing concomitant systemic infections (gastroenteritis, pneumonia, malaria, tuberculosis) which impair vitamin A

Table 7. Recommended xerophthalmia treatment schedule—oil-miscible oral vitamin A

	< 1 year of age	≥ 1 year of age*
Immediately	100,000 IU	200,000 IU
The next day	100,000 IU	200,000 IU
2–4 weeks later	100,000 IU	200,000 IU
	Severe protein energy malnutrition (PEM)	
Monthly until PEM resolves	100,000 IU	200,000 IU

*Women of reproductive age require special attention because of the potential teratogenic effects of very high dose retinol early in pregnancy. As corneal lesions are a severe, medical emergency, apply the full therapeutic regimen described above. If the woman is otherwise healthy and only suffering from nightblindness or Bitot's spots, treat instead with daily doses of 10,000 IU orally for 2 weeks.

metabolism and increase its utilization; and reversing dehydration. Any evidence of corneal infection should be dealt with by intensive topical and systemic therapy.

One must be careful not to be misled into ruling out vitamin A deficiency because the eye is inflamed or lacks classical Bitot's spots; or administer oily preparations of vitamin A parenterally (Sommer, 1989). If the only vitamin A preparation available is injectable, oily vitamin A, remove the needle and squirt the vitamin into the mouth. A far larger proportion will be absorbed.

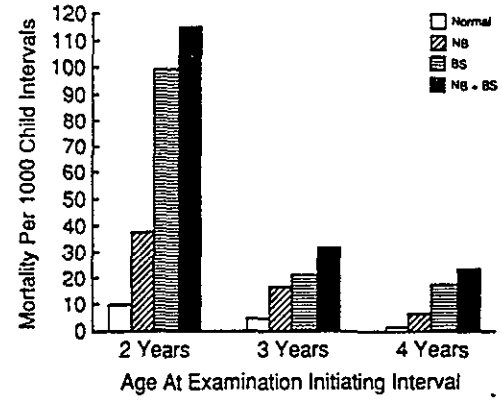


Fig. 7. Mortality per 1000 child-intervals among children who were 2 to 4 years of age (at the first of their interval-initiating rounds). All children with respiratory disease at an interval-initiating round have been excluded. (From Sommer *et al.*, 1983.)

5. SYSTEMIC MORBIDITY AND MORTALITY

Most of the scientific and policy interest in vitamin A deficiency of the past 15 years has been centered not on the eye (xerophthalmia) but on newly (rediscovered) systemic morbidity and mortality. Since vitamin A is responsible for normal differentiation of cells affecting organs throughout the body, it's not surprising that deficiency should cause more than ocular changes. While systemic changes are not the prime interest of this publication, the area needs to be dealt with, even if briefly. A detailed account is available elsewhere (Sommer and West, 1996).

Despite early animal experiments and clinical observations, for years medical interest focused solely on the ocular manifestations of deficiency (xerophthalmia). A marked change in attitude began when children, considered to be suffering mild vitamin A deficiency (XN, X1B, or the two combined), were found to die at higher rates than their peers; and that the risk of death increased with the severity of their deficiency (Fig. 7) (Sommer *et al.*, 1983).

An initial randomized prophylaxis trial, in which half the 22,000 preschool children in 450 villages were assigned to receive a 200,000 IU vitamin A supplement every 6 months, demonstrated reduction in child mortality rates of 34–70% (Sommer *et al.*, 1986; Sommer and Zeger, 1991). This was followed by seven additional trials (Muhilal *et al.*, 1988; West *et al.*, 1991; Daulaire *et al.*, 1992; Rahmathullah *et al.*, 1990; Vijayaraghavan *et al.*, 1990; Herrera *et al.*, 1992; Ghana VAST Study Team, 1993) in different countries and cultures, utilizing a myriad of strategies for improving vitamin A status (periodic large doses every 4–6 months, weekly doses, fortification of a commonly consumed product). Results were remarkably similar (Fig. 8) except for two studies (Vijayaraghavan *et al.*, 1990; Herrera *et al.*, 1992), which subsequent analysis revealed to have suffered problems of quality control (Sommer and West, 1996). Results of the others were remarkably consistent: a marked reduction in overall child mortality among those assigned to receive oral vitamin A supplementation. Even including the two 'outlier' studies, meta-analysis

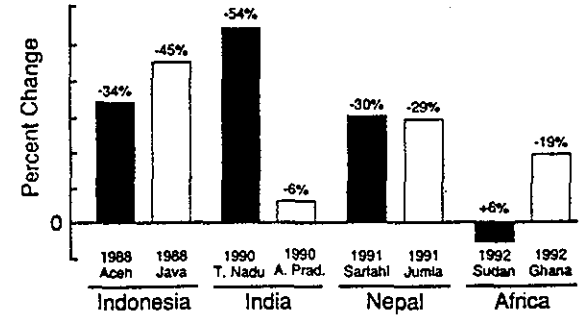


Fig. 8. Difference in preschool mortality (6 or 12 months to 6 years of age) in 8 major community-based vitamin A prophylaxis trials. All figures as reported on an 'intent to treat' basis showing proportional differences in mortality for the vitamin A group. There was a net reduction in mortality among the vitamin A group in all trials but the Sudan.

Table 8. Cause-specific mortality, vitamin A supplementation community prevention trials

	Symptoms/diseases					
	Measles		Diarrhea		Respiratory	
Study	Vitamin A	Control	Vitamin A	Control	Vitamin A	Control
Tamil Nadu[2] (Rahmathullah *et al.*, 1990)						
Deaths (*n*)	7	12	16	33	2	3
RR*	0.58		0.48		0.67	
NNIPS[1] (West *et al.*, 1991)						
Deaths (*n*)	3	12	39	62	36	27
RR	0.24		0.61		1.29/1.00†	
Jumla (Daulaire *et al.*, 1992)						
Deaths (*n*)	3	4	94	129	18	17
RR	0.67		0.65		0.95‡	
Ghana[3] (Ghana VAST Study Team, 1993)						
Deaths (*n*)	61	72	69	111	47	45
RR	0.82		0.66§		1.00	

*RR (Relative Risk): Cause-specific mortality rate of vitamin A group divided by rate in control group.
†Original published results (West *et al.*, 1991): RR = 1.29; re-analysis as an associated cause that recognizes underlying causes; RR = 1.00 (Vitamin A and Pneumonia Working Group, 1995).
‡Pneumonia case management program may have confounded results.
§Defined as 'acute gastroenteritis'.
‖Except for Jumla, findings relate to children already ≥ 6 months of age when supplemented.

confirmed a strong, positive impact on preschool child survival associated with improvement in vitamin A status (Beaton *et al.*, 1992).

Four studies examined cause-specific mortality. Each concluded that the greatest reduction was in measles and diarrhea-related case fatality (Table 8) (Rahmathullah *et al.*, 1990; West *et al.*, 1991; Daulaire *et al.*, 1992; Ghana VAST Study Team, 1993). The magnitude of the reduction in measles mortality in these prophylaxis trials is almost identical to the reduction in case-fatality rates in the treatment trials of children already hospitalized with moderate to severe measles (Barclay *et al.*, 1987; Ellison, 1932; Hussey and Klein, 1990). This strongly suggests high-dose vitamin A treatment of measles, now a recommendation of WHO and UNICEF (World Health Organization, 1987) as well as the American Academy of Pediatrics (Committee on Infectious Diseases, 1993), exerts its effect by improving vitamin A status and not, as some have suggested, by a non-specific adjuvant effect on the immune system by the large dose used (Keusch, 1990). Otherwise, the effect on the community prophylaxis trials, where dosing occurred long before the measles occurred, would have had a much smaller (if detectable) impact. Treatment of measles with vitamin A will not only reduce mortality but blindness as well.

There is relatively little evidence that vitamin A status affects the incidence of infection; rather it seems to modify its severity. Measles cases treated with vitamin A suffered fewer subsequent severe complications, protection extending as long as 6 months following the acute attack (Barclay *et al.*, 1987; Hussey and Klein, 1990; Coutsoudis *et al.*, 1991; Coutsoudis *et al.*, 1992). In an even more generic study, prophylactic, large-dose vitamin A (or placebo) was delivered every 4 months to Ghanian children who were followed for systemic morbidity. Severe diarrhea, all-cause clinic attendance and hospitalization rates among vitamin A recipients was reduced by 10–32% (Ghana VAST Study Team, 1993).

The mechanism(s) by which vitamin A status imparts its effects are as yet unclear. Presumably the abnormal keratinized epithelial linings that exist with vitamin A deficiency provide a less effective barrier to infection than the (ciliated) mucous-secreting epithelium normally present. It's also clear that improvement in vitamin A status increases both humoral and cell-mediated immunity, whether to test antigens or natural measles infection (Coutsoudis *et al.*, 1992; Semba *et al.*, 1992).

As befitting the apparent importance of vitamin A in mounting an immune response, there is a growing body of evidence that vitamin A status

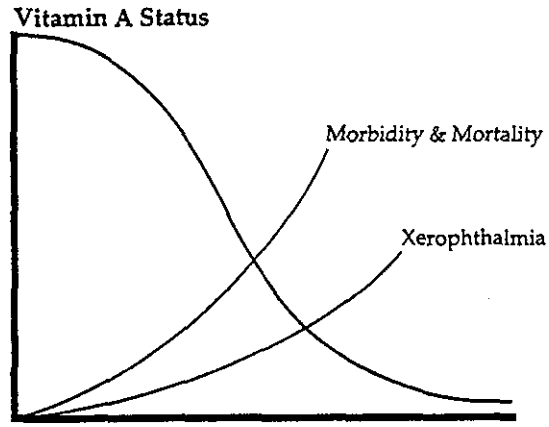


Fig. 9. Stylized relationship between the rise in systemic and ocular consequences associated with a fall in vitamin A status.

may exert a profound effect on a variety of HIV parameters, including longevity and vertical transmission (Semba *et al.*, 1993; Semba *et al.*, 1994; Coutsoudis *et al.*, 1995).

In addition to mortality and severity of infectious episodes, vitamin A deficiency also interferes with the utilization of iron, producing or aggravating anemia (Muhilal *et al.*, 1988; Mejia and Chew, 1988). It also affects growth, but in ways made complex by the adequacy of other growth-limiting nutrients.

Vitamin A deficiency begins to alter other health indices long before it becomes manifest as clinically recognizable xerophthalmia (Fig. 9). If this were not the case, supplementation would not have the dramatic impact it does on case-fatality rates of non-xerophthalmic children hospitalized with measles. Nor would prophylactic supplementation with vitamin A reduce preschool mortality to the degree that it does: we estimated that elimination of all xerophthalmia from Indonesia would reduce overall preschool-age childhood mortality by 16% (Sommer *et al.*, 1983); instead, supplementation reduced mortality by at least twice that amount. A ready explanation is at hand. Vitamin A levels of non-xerophthalmic children were, on average, less than 20 μg/dl (when truly normal levels are 30 μg/dl or more) (Sommer *et al.*, 1980c). Our original estimation of the increased mortality among children with 'mild xerophthalmia' (XN, X1B) was based on a comparison with non-xerophthalmic children from the same villages (RR = 1); yet these non-xerophthalmic children, with their suboptimal vitamin A status, were probably experiencing unnecessarily increased mortality themselves. Their increased risk of mortality was no doubt lower than for those with more severe deficiency (XN, X1B), but they constituted a much larger portion of the population. Ancillary studies of the physiologic function of these children support this point: only 3% of all children had evidence of xerophthalmia, but over 30% had abnormal impression cytology (Natadisastra *et al.*, 1988), strongly suggesting that xerophthalmia is a late manifestation of severe deficiency, while other abnormalities begin to occur at earlier, milder stages. The risk of physiologic abnormality, and its severity, increases with increasing deficiency. From data like these, we can estimate that improved vitamin A status would prevent five to ten million cases of xerophthalmia (Sommer *et al.*, 1981b), one to three million deaths, and the subtler consequences faced by the 124 million deficient children each year (Humphrey *et al.*, 1992).

It is now clear that improving the vitamin A status of children around the world will both prevent blindness and reduce systemic morbidity and mortality. This has important policy implications. Whereas governments were previously loathe to divert limited health resources 'merely' to prevent xerophthalmia and its associated blindness ("when 20% to 40% of our children die before the age of five"), these new insights have provided justification and political will for doing just that.

6. FUTURE DIRECTIONS

Research issues related to vitamin A deficiency cover a wide canvas, stretching from basic mechanisms at the heart of corneal melting and retinol regulation of gene expression to devising practical approaches for improving vitamin A status protecting the sight and lives of millions of people every year. In an unusual confluence of time and disease, we are scientifically challenged at both extremes at the same time. Indonesia demonstrates this is no illusion. The Ministry of Health and its research arms collaborated in providing many

of the new insights of the past two decades recorded in this chapter. As a result, the government accepted the validity of the findings and committed itself to acting upon its implications. Mounting a multi-pronged effort to improve the vitamin A status of its citizens, Indonesia virtually eliminated xerophthalmia as an important cause of pediatric blindness (Muhilal *et al.*, 1994). While not everyone's vitamin A status is now normal, which means that many remain at increased risk of systemic morbidity and mortality, deficiency is less prevalent and severe than previously. But if the government persists in its approach—increasing the intake of vitamin A-rich foods, providing large-dose periodic supplements where the combination of diet and environment keep people at risk—or, like the Philippines, finally identifies a dietary item consumed by most of the high risk group suitable for vitamin A fortification, the problem can be eliminated entirely, just as it was from wealthier countries like Denmark, Singapore, and Malaysia. Importantly, Indonesia continues its research efforts despite having achieved its initial goal, because it recognizes that today's research provides (more effective) answers to tomorrow's problems.

6.1. Research Needed to Reduce the Prevalence and Severity of Deficiency

Among the greatest constraints to widespread intervention programs is the lack of a simple, definitive technique for assessing individual and population-wide vitamin A status under rigorous field conditions. While new, more practical techniques have become available, simpler, more definitive ones are still needed. Ideally these would provide data on two important aspects of vitamin A status. The first is the adequacy of physiologic functions dependent upon vitamin A, demonstrating that sufficient vitamin A is available to target tissues to meet present demands. It would be helpful to distinguish the cause of any inadequacy identified: whether due to inadequate stores of vitamin A, deficiencies in protein-related delivery mechanisms, or the lack of co-factors required for normal metabolism (e.g. zinc). The second approach would measure adequacy of vitamin A stores needed to prevent deficiency if intake were to drastically decline or metabolic demand (from infection or the like) increase. This was the theoretical basis upon which Olson judged that liver concentrations of 20 μg/g were 'adequate': by estimating such liver stores could provide sufficient vitamin A to support key physiologic functions for 4 months, even if all intake ceased (Olson, 1987). This is the surest means of estimating the sufficiency of vitamin A as a limiting factor and the elasticity of safe intake. Assessment is the key to identifying populations in need of intervention and the success those interventions have had.

Assessment is primarily in support of programmatic intervention. Large-dose capsule supplementation, so effective under controlled research conditions, is not a long-term sustainable solution to inadequate dietary intake. While the capsules themselves are inexpensive, delivering them to those most in need is not. Regions in which vitamin A deficiency is most severe lack the underlying health infrastructure and cultural practices that would otherwise provide an inexpensive mechanism for administering the supplement (e.g. prenatal maternal care, postnatal routine exams, etc.). Furthermore, it is a general rule of thumb that those most in need of healthy interventions (whether a change in diet, immunization, or receipt of capsules) are the least compliant. It helps to ensure that adequate capsules are freely available for use at health centers, dispensaries and the like, and to incorporate large-dose capsule distribution as part of other outreach programs (e.g. Expanded Program for Immunization). But it is too expensive and inefficient to maintain a dedicated individual delivery mechanism. Entirely different tacks may be taken: for example, using social marketing to encourage mothers to take and provide their children with a smaller daily (or weekly) dose of vitamin A that meets their needs and precludes concerns over toxicity and teratogenic potential (unless recommendations are grossly exceeded). Such capsules could be made widely available at low cost at all pharmacies or markets.

A change in diet has long been held as the ultimate solution, and no doubt increased consumption is the reason vitamin A deficiency eventually disappeared from wealthier countries.

4. SPECIAL SESSION

Steven C. LeClerq, Rapporteur

Vitamin A for Health, Vision and Survival

Presenter: Dr. Keith P. West, Jr., Professor, Dept. of International Health, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, U.S.A.

Dr. West discussed the Vitamin A deficiency problem and its possible solutions in Nepal in the context of the work of the Nepal Nutrition Intervention Project - Sarlahi (NNIPS), which is a joint project of Johns Hopkins and Nepal Netra Jyoti Sangh (NNJS), with assistance from the Sushil Kedia Seva Mandir in Sarlahi, under the auspices of the Social Welfare Council and with the approval of the Nepal Health Research Council of His Majesty's Government of Nepal. NNIPS is funded by USAID and receives additional support from Task Force Sight and Life of Roche, Switzerland.

Vitamin A deficiency (VAD) has multiple effects in the body. These can include the development of night blindness, xerophthalmia and more serious eye problems, sometimes leading to blindness. Mild immune suppression, anemia, loss of ciliated, mucous-secreting epithelia lining the respiratory, gastro-intestinal, genito-urinary tracts and glandular ducts and loss of brush border and goblet cells in the intestine all contribute to increased susceptibility to infection and decreased ability to successfully weather bouts of severe infection. Squamous metaplesia of the intestinal lining is also a possible consequence of VAD. A vitamin A xerophthalmia study in Aceh province of Indonesia observed a significant reduction in preschool child mortality among children dosed with twice-yearly large-dose vitamin A compared to those who received a placebo. These intriguing results needed to be replicated in a study designed specifically to look at the possible effect of periodic vitamin A dosing on preschool child mortality and survival.

National blindness surveys in Nepal indicated that vitamin A deficiency, clinically manifested as xerophthalmia, is a significant public health problem in many areas of the country. The east-central Tarai has especially high levels of xerophthalmia. Consequently, in coordination with NNJS, it was decided to carry out a large community trial (38,000 children) of the efficacy of vitamin A in reducing preschool child mortality in the east-central Terai district of Sarlahi (NNIPS-1). Vitamin A was found to reduce child mortality by 30%. These findings coupled with similar findings from concurrent studies in several other countries provided the basis for the establishment of the Nepal National Vitamin A Program (NVAP). As of autumn, 1998, children in 47 districts of Nepal will have been receiving the benefits of twice-yearly vitamin A dosing and nutrition education. It is estimated that at least 25,000 child deaths are being averted each year through the receipt of large-dose vitamin A capsules by children. The NVAP is slated to cover all 75 districts of Nepal by the autumn of the year 2001. The NNIPS-1 research results leading to the establishment of the NVAP is a perfect example of relevant public health research being translated into beneficial action by motivated policy makers and program planners.

It is known from the Aceh and NNIPS research that xerophthalmia and other clinical signs of vitamin A deficiency represent only the tip of the iceberg of subclinical vitamin A deficiency (VAD), and that children with subclinical VAD experience a higher risk of dying than those without this condition. Increasing severity of clinical signs of VAD is associated with accordingly increasing risk of mortality, as well. This understanding, coupled with the knowledge from the NNIPS-1 research results that xerophthalmia and VAD cluster, can be used as an effective public health tool to identify high risk communities and households for intervention. The presence of one xerophthalmic child in a community identifies the other children in that community as being twice as likely to be Vitamin A deficient than children in communities without xerophthalmic children. The presence of a xerophthalmic child in a household identifies the other siblings in that household as being at a 7 to 13 times higher risk of experiencing VAD and its accompanying morbidity and mortality risks.

The NNIPS child mortality reduction results were not seen in infants under six months of age. At the same time, it was observed that almost 20% of women in the NNIPS study area were reporting night blindness during pregnancy, indicating a significant VAD problem not only among children, but among adult women as well. These findings provided the basis for the second large community trial (NNIPS-2), which evaluated the effect of dietary amounts of vitamin A and its precursor, beta-carotene, in reducing maternal mortality, stillbirth, miscarriage and early infant death. In this trial, 45,000 women were visited every week to be dosed with their assigned coded capsule, asked about menstrual activity during the past week, and about their pregnancy status (and eventual outcome). More than 22,000 pregnancies resulted from this population of women over the course of the study. It was found that maternal mortality was reduced by 40% with vitamin A, compared to the placebo group. Beta-carotene reduced maternal mortality by 49%, for a combined effect of 44% reduction in maternal mortality with Vitamin A or beta-carotene (strongly statistically significant). These reductions in mortality were

found across all different causes of maternal death. Thus, the indications are that increasing vitamin A intake, either preformed or as beta-carotene, among women of child-bearing age may substantially increase maternal survival during and following pregnancy.

These results are of particular significance and relevance when the risk of dying in different age and sex groups in Nepal is considered relative to the risk experienced by similar groups in a western country such as the U.S. Infants in Nepal are at an almost 11 times higher risk of dying than are infants in the U.S., while under-five children in Nepal are at a 14 times increased risk of dying. But even more striking and disturbing is the data for relative risk of maternal mortality, which shows that women in Nepal are at an almost 70 times higher risk of dying due to pregnancy and childbirth-related causes than are women in the U.S., indicating a public health problem of huge proportions, which demands concerted efforts to find solutions.

Ethnographic studies conducted as part of NNIPS have shown that VAD is also associated with a syndrome of less than optimal child care. Compared with non-xerophthalmic children, those with xerophthalmia were not fed "automatically" at nearly the same rate by their parents. As a result, they would have to serve themselves more often. Such children are also scolded and hit more frequently and receive less positive health behaviors, such as nit-picking, face-washing, etc. This evidence in conjunction with the NNIPS maternal mortality reduction results helps in constructing a picture of family and community Vitamin A Deficiency Syndrome where the Vitamin A deficient fetus develops into a VAD infant and child, then a VAD adolescent and ultimately a VAD adult. This syndrome is most serious in women who then are more likely to give birth to VAD children and be at higher risk of death themselves.

A sub-study within NNIPS-2 showed that 76% of the women in Sarlahi are anemic and about the same proportion are infested with hookworm. Vitamin A was seen to improve hemoglobin levels in women with no or low levels of hookworm parasitemia. This finding gives credence to periodic deworming efforts for women of child-bearing age. Thus, it has become clear that during the child-bearing years, women pay a high price for being chronically vitamin A deficient. They become vitamin A-depleted in late pregnancy, develop night blindness, suffer a high burden of morbidity and anemia and are at higher risk of dying during and following pregnancy. NNIPS found that a simple history of night blindness during pregnancy identifies women who are more vitamin A deficient, chronically and acutely malnourished, anemic and generally ill. Nightblind women were found to be at approximately a two-times higher risk of morbidity such as urinary tract infection, diarrhea and dysentery, toxemia of pregnancy, nausea and vomiting than women without night blindness. They were also an amazing 7.5 times higher risk of dying. These findings show that the recognition of night blindness during pregnancy can be another valuable public health tool which allows health workers to identify women at high risk of various morbidity and especially death, thus creating opportunities for intervention.

Program and policy recommendations emerging from NNIPS-2 point to the importance of assuring a diverse diet adequate in beta-carotene and vitamin A for all women. Possible antenatal/perinatal supplementation of women can also be considered. The importance of identifying and preventing maternal nightblindness and its accompanying risks also cannot be over-emphasized. Preventing hookworm infestation to enhance the impact of vitamin A and beta-carotene on reducing anemia has also been emphasized.

Group Discussion

A comment was made to the effect that setting national health research agenda priorities with the expectations of the people in mind is a truly laudable goal, but needs to be tempered with the real fact that some major health problems are masked to the people, simply by the fact of their being so commonplace as to be considered normal. In this respect, it is truly appropriate to refer to vitamin A and other micronutrient deficiencies as "Hidden Hunger".

A pertinent example is that of vitamin A deficiency, which until the recent emergence of the National Vitamin A Program, remained unknown as a problem to most village people of Nepal. Yet, when rigorous public health research was conducted on a very relevant topic identified by public health professionals, it was found that vitamin A deficiency is indeed a serious public health problem in Nepal, and that it can be remedied to a large degree in children through a twice-yearly mass dosing campaign approach, while ongoing efforts are underway to educate parents about the importance of feeding more vitamin A-rich foods to their children and to women in the household, as well as producing more at the household level. The challenge of creating a similarly effective solution to the VAD problem among women remains to be taken up by public health workers everywhere. Once reliable knowledge is in hand, the task remaining is to advocate the use of knowledge to stimulate change, exchange information and ideas with coworkers and colleagues in various fields, identify gaps in knowledge and attempt to fill them, communicate findings to wide audiences and, most importantly, act on existing knowledge to benefit the largest number of people possible.

सचेतना

SACHETANA

JOURNAL OF ESSENTIAL NATIONAL HEALTH RESERCH NEPAL

123

VITAMIN A AND HIV

INT J TUBERC LUNG DIS 2(9):771–773
© 1998 IUATLD

SHORT COMMUNICATION

Serum vitamin A levels during tuberculosis and human immunodeficiency virus infection

J.-M. Rwangabwoba,* H. Fischman,† R. D. Semba‡

*Centre Universitaire de Santé Publique, Butare, Rwanda, †Department of Epidemiology, School of Hygiene and Public Health, ‡Department of Ophthalmology, School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA

SUMMARY

Vitamin A deficiency during tuberculosis and human immunodeficiency virus (HIV) infection has not been characterized. A cross-sectional study was conducted among HIV-infected adults with tuberculosis in Butare, Rwanda, in which 29% of the subjects had serum vitamin A levels consistent with deficiency (<1.05 μmol/L). Women had mean serum vitamin A levels of 1.22 ± 0.45, compared with 1.47 ± 0.68 in men ($P < 0.07$). A total of 37% of subjects with recent weight loss had vitamin A levels consistent with deficiency, compared with 14% of subjects without weight loss ($P < 0.02$). This study suggests that vitamin A deficiency is common among adults with tuberculosis and HIV infection in Rwanda.

KEY WORDS: HIV; nutrition; Rwanda; tuberculosis; vitamin A

VITAMIN A is essential for normal function of T and B lymphocytes, generation of antibody responses, and maintenance of mucosal surfaces of the respiratory, gastrointestinal, and genitourinary tracts.[1] In developing countries, vitamin A deficiency is a leading cause of morbidity and mortality.[1] Recent studies suggest that vitamin A supplementation may reduce young child and maternal mortality.[1,2] Vitamin A deficiency has not been described in patients co-infected with tuberculosis and human immunodeficiency virus (HIV) infection. We conducted a cross-sectional study to determine the prevalence of vitamin A deficiency in adults infected with HIV and tuberculosis in Rwanda.

STUDY POPULATION AND METHODS

The study population consisted of a consecutive sample of HIV-infected adults admitted with tuberculosis to the Hôpital Universitaire de Butare, Hôpital de Kabutare, or Hôpital de Nyanza, in Butare, Rwanda, between 31 September 1992 and 15 January 1994. Subjects were considered HIV-infected on the basis of a positive HIV enzyme immunoassay (EIA Vironostika, Organon-Teknika, Boxtel, The Netherlands) with confirmation by Western blot (Biotech, Du Pont de Nemours, Wilmington, DE, USA). Pulmonary tuberculosis was diagnosed on the basis of a sputum sample from three non-induced sputum samples positive for acid-fast bacilli, and extra-pulmonary tuberculosis was diagnosed on the basis of pleural or cerebrospinal fluid (CSF) positive for acid-fast bacilli by histopathology and/or by culture. Cultures were routinely taken on pleural and CSF samples.

All subjects received 5 TU of purified protein derivative (PPD) 0.1 mL, and 40 colony-forming units (cfu)/mL mumps antigen 0.1 mL, delivered intradermally on the volar aspect of the left forearm, and results of anergy skin testing were read 48 hours later. A positive tuberculin test result was defined as induration ≥5 mm, and a mumps test result was considered positive if the induration was ≥2 mm. A standardized morbidity history was obtained at the time of admission. Bacille Calmette-Guérin (BCG) immunization is national policy in Rwanda, and subjects were not examined for a BCG scar. Serum samples were obtained upon admission and stored in a sample repository at −70°C until analysis. Serum vitamin A levels were measured by high performance liquid chromatography (HPLC)[3] at Johns Hopkins Hospital using reference standards from the National Institute of Standards and Technology (Gaithersburg, MD, USA). $CD4^+$ lymphocyte counts were measured by flow cytometry (FACScan, Becton Dickinson Immunocytometry, San Jose, CA, USA)[4] in the Johns Hopkins University/Université National de Rwanda Prorecherche Laboratory in Butare.

Student's *t*-test and Wilcoxon nonparametric tests were used for comparisons of continuous data, and appropriate variable transformations were made for

Correspondence to: Dr Richard Semba, Ocular Immunology Service, Suite 700, 550 North Broadway, Baltimore, MD 21205, USA. Tel: (+410) 955-3572. Fax: (+410) 955-0629. e-mail: rdsemba@welchlink.welch.jhu.edu

125

skewed data. Serum vitamin A levels <1.05 μmol/L are considered to be consistent with deficiency in adults.[1,4] Categorical analyses were done with χ^2 analyses and exact tests. Means and standard errors are reported for continuous variables.

RESULTS

Serum vitamin A levels were measured in 94 HIV-infected adults with tuberculosis (33 women and 61 men, mean age 31.7 ± 6.9 and 34.4 ± 8.5, respectively). Mean serum vitamin A level was 1.38 ± 0.61 overall, and 27 of 94 subjects (29%) had serum vitamin A levels consistent with vitamin A deficiency (<1.05 μmol/L). The coefficient of variation in serum vitamin A measurements by HPLC was 4.5%. The frequency distribution of serum vitamin A levels is shown in the Figure. The mean CD4+ lymphocyte count was 272 ± 228, and 31% of adults had CD4+ lymphocyte count <200 cells/L.

The relationships between age, sex, CD4+ count, tuberculosis site, and skin anergy testing and serum vitamin A levels were examined as shown in the Table. These data suggest that lower serum vitamin A levels are found among women than men, and among those with negative mumps skin testing. Vitamin A levels <1.05 μmol/L were found in 37% of subjects reporting recent weight loss at admission, compared with 14% of subjects who reported no recent weight loss ($P < 0.02$), but there was no significant relationship between a recent history of diarrhea, fever, or cough with lower serum vitamin A levels.

DISCUSSION

This study suggests that vitamin A deficiency is common among HIV-infected adults co-infected with tuberculosis. To our knowledge, this is the first study to examine the prevalence of vitamin A deficiency in adults infected with tuberculosis and HIV. Factors which may contribute to low serum vitamin A levels include poor dietary intake of vitamin A-containing foods, malabsorption, diarrhea, liver disease, protein-energy malnutrition, zinc deficiency, the acute phase response, and abnormal vitamin A losses in the urine during infections.[5] It is likely that several factors contribute to the high prevalence of vitamin A deficiency in this study population. A limitation of the study is that serum vitamin A levels in healthy control adults were not measured, however the frequency distribution of serum vitamin A levels from other populations are available for comparison.[1,5]

During the acute phase response, vitamin A levels may drop because of impaired ability of the liver to mobilize vitamin A and its carrier protein complex which consists of retinol-binding protein and transthyretin. This has led to concern that serum vitamin A levels during infection may not be an accurate reflection of hepatic vitamin A stores. However, it should be noted that during tuberculosis infection, low serum vitamin levels, depleted liver stores of vitamin A, and clinical vitamin A deficiency, i.e., nightblindness, have been described.[6,7] In addition, a comparison of the frequency distributions of serum vitamin A levels in large population-based studies of HIV-infected adults suggests that circulating vitamin A levels reflect the vitamin A status of populations.[5]

The present study suggests that individuals with weight loss have significantly lower vitamin A levels. Recent weight loss is common in adults with active tuberculosis, and the anorexia associated with infection could reduce both protein and vitamin A intake. Anergy to mumps antigen was associated with lower vitamin A levels and may be related to depressed immunity in vitamin A deficiency.[1] Delayed type hypersensitivity reactions have been shown to be

Table Characteristics of HIV-infected adults with tuberculosis and serum vitamin A levels

Characteristic	*n*	Serum vitamin A (mean ± SEM)	*P* value
Age (years)			
<35	58	1.41 ± 0.60	0.57
≥35	36	1.34 ± 0.63	
Sex			
Female	33	1.22 ± 0.45	0.07
Male	61	1.47 ± 0.68	
CD4+ lymphocyte count			
<200 cells/ L	39	1.41 ± 0.70	0.79
≥200 cells/ L	44	1.37 ± 0.59	
Tuberculosis site			
Pulmonary	69	1.42 ± 0.63	0.24
Extra-pulmonary	25	1.33 ± 0.55	
PPD skin test			
Positive	35	1.40 ± 0.49	0.74
Negative	34	1.44 ± 0.75	
Mumps skin test			
Positive	16	1.74 ± 0.72	0.01
Negative	53	1.32 ± 0.57	

SEM = standard error of mean; PPD = purified protein derivative.

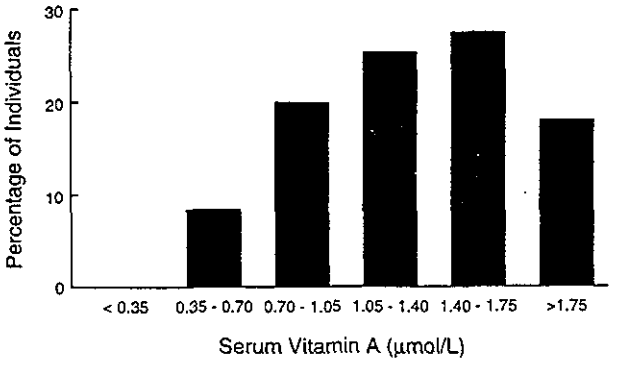


Figure Frequency distribution of serum vitamin A levels among 94 adults with tuberculosis and HIV infection in Butare,

126

which may be related to vitamin A-related upregulation of lymphocyte and/or Langerhans' cell function.[9]

The results of the present study suggest that lower vitamin A levels are found in women, as has been previously described in larger population-based studies elsewhere. It is unclear why women of childbearing age are at higher risk for vitamin A deficiency than men, however both pregnancy and lactation increase the risk of vitamin A deficiency.[1] Further studies of dietary assessment at the household level are needed to determine whether the gender differences in vitamin A status are due to dietary and behavioral factors.

It is unknown whether vitamin A supplementation will reduce mortality in HIV-infected adults. Cod-liver oil, one of the richest sources of vitamin A, was used for over one hundred years as a main treatment for tuberculosis, prior to the development of antibiotic therapy. Tuberculosis is undergoing a global resurgence, largely due to the AIDS pandemic, and by the year 2000 it is estimated that about 13% of HIV-infected individuals will be coinfected with tuberculosis.[10] In spite of adequate chemotherapy for tuberculosis, mortality rates in HIV-infected adults in developing countries remain extremely high, and whether vitamin A supplementation could improve clinical outcomes for these individuals is not known.

Acknowledgements

We thank Bruno Ngirabarware, Emmanuel Uwimbabazi, and Célestin Higiro for the collection of clinical data on the study participants, Marc Bulterys and Lew Barker for reviewing the manuscript, and Ann L Cook for administrative support.

This study was supported by the Fogarty International Training Program in AIDS and Epidemiology, the National Institutes of Health (HD30042, HD32247), and the United States Agency for International Development (Cooperative Agreement DAN-0045-A-5094-00).

References

1 Sommer A, West K P Jr. Vitamin A deficiency: health, survival, and vision. New York: Oxford University Press, 1996.
2 West K P Jr, Khatry S K, Katz J, et al. Impact of weekly supplementation of women with vitamin A or beta-carotene on fetal, infant and maternal mortality in Nepal. Abstracts of the 18th International Vitamin A Consultative Group Meeting, Cairo, Egypt, 22–26 September, 1997. Washington, DC. Human Nutrition Institute, 1997.
3 Semba R D, Graham N M H, Caiaffa W T, Margolick J B, Clement L, Vlahov D. Increased mortality associated with vitamin A deficiency during human immunodeficiency virus type 1 infection. Arch Intern Med 1993; 153: 2149–2154.
4 Giorgi J V, Cheng H L, Margolick J B, et al. Quality control in the flow cytometric measurement of T-lymphocyte subsets: the Multicenter AIDS Cohort Study experience. Clin Immunol Immunopathol 1990; 55: 173–186.
5 Semba R D. Vitamin A and human immunodeficiency virus infection. Proc Nutr Soc 1997; 56: 1–11.
6 Moore T, Sharman I M. Vitamin A levels in health and disease. Brit J Nutr 1951; 5: 119–129.
7 Harris R, Harter J S. Night blindness and vitamin A deficiency in pulmonary tuberculosis. South Med J 1940; 33: 1064–1065.
8 Rahman M M, Mahalanabis D, Alvarez J O, Wahen M A, Islam M A, Habte D. Effect of early vitamin A supplementation on cell-mediated immunity in infants younger than 6 mo. Am J Clin Nutr 1997; 65: 144–148.
9 Semba R D. The role of vitamin A and related retinoids in immune function. Nutr Rev 1998; 56: S38–S48.
10 Dolin P J, Raviglione M C, Kochi A. Global tuberculosis incidence and mortality during 1990–2000. Bull World Health Organ 1994; 72: 213–220.

RÉSUMÉ

L'on n'a pas défini jusqu'ici la déficience en vitamine A au cours de la tuberculose et de l'infection par le virus de l'immunodéficience humaine (VIH). Nous avons mené une étude transversale chez des adultes infectés par le VIH et atteints de tuberculose à Butare, Rwanda. Les niveaux de vitamine A sérique étaient compatibles avec une déficience (<1,05 μmol/L) chez 29% des sujets. Les niveaux moyens de vitamine A sérique étaient de 1,22 ± 0,45 chez les femmes et de 1,47 ± 0,68 chez les hommes ($P < 0,07$). Des niveaux de vitamine A allant de pair avec une déficience se retrouvent chez 37% des sujets avec perte de poids récente (contre 14% chez les sujets sans perte de poids récente : $P < 0,02$). Cette étude suggère que la déficience en vitamine A est courante chez les adultes atteints de tuberculose et d'infection par le VIH au Rwanda.

RESUMEN

No es bien conocida la deficiencia de la vitamina A en la tuberculosis y en la infección por el virus VIH. Se efectuó un estudio transversal en pacientes con tuberculosis e infectados por el VIH en Butare, Ruanda. El 29% de los enfermos tenían niveles séricos de vitamina A compatibles con una deficiencia (<1,05 μmol/L). Las mujeres presentaron un nivel promedio de vitamina A de 1,22 ± 0,45 y los hombres un nivel de 1,47 ± 0,68 ($P < 0,07$). El 37% de los pacientes con una reciente pérdida de peso tenían niveles de vitamina A deficientes, comparados con el 14% que no habían perdido peso ($P < 0,02$). Este estudio sugiere que la deficiencia de vitamina A es común en los adultos con tuberculosis e infección por VIH en Ruanda.

Vitamin A Supplementation and Human Immunodeficiency Virus Load in Injection Drug Users

Richard D. Semba, Cynthia M. Lyles, Joseph B. Margolick, Waleska T. Caiaffa, Homayoon Farzadegan, Sylvia Cohn, and David Vlahov

Department of Ophthalmology, Johns Hopkins School of Medicine, and Departments of Epidemiology and Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland

The use of vitamin A therapy during human immunodeficiency virus (HIV) infection is under clinical investigation, and vitamin A could potentially modulate HIV replication because the virus genome contains a retinoic acid response element. A randomized, double-masked, placebo-controlled clinical trial was conducted to determine the impact of single high-dose vitamin A supplementation, 60-mg retinol equivalent (200,000 IU), on HIV load and CD4 lymphocyte count. HIV-infected injection drug users (120) were randomly allocated to receive vitamin A or placebo. Plasma vitamin A level, CD4 lymphocyte count, and HIV load were measured at baseline and 2 and 4 weeks after treatment. Vitamin A supplementation had no significant impact on HIV load or CD4 lymphocyte count at 2 and 4 weeks after treatment. This study suggests that high-dose vitamin A supplementation does not influence HIV load.

Micronutrient deficiencies and wasting are common during human immunodeficiency virus (HIV) infection [1, 2]. Low intake of some micronutrients, such as vitamin A, has been associated with increased progression to AIDS [3]. Vitamin A and its active metabolites are necessary for normal T and B cell function, macrophage function, antibody responses to protein antigens, and maintenance of respiratory and gastrointestinal mucosal integrity [4, 5]. Epidemiologic studies suggest that low serum or plasma vitamin A level is an independent risk factor for mortality during HIV infection [6, 7] and that low vitamin A levels during pregnancy may contribute to increased mother-to-child transmission of HIV [8]. Among HIV-infected lactating women in Kenya, low vitamin A levels have been linked to higher HIV loads in breast milk, suggesting that maternal vitamin A status may influence HIV transmission through breast-feeding [9]. These epidemiologic studies suggest a potential role of vitamin A supplementation in improving clinical outcome during HIV infection. Two recent clinical trials of vitamin A supplementation in HIV-infected children suggest that vitamin A reduces infectious disease morbidity [10] and increases circulating NK and CD4 cell counts [11].

The active metabolite of vitamin A, all-*trans* retinoic acid, regulates gene expression via nuclear receptors known as retinoic acid receptors (RAR) and retinoid-X receptors (RXR) [12]. All-*trans* retinoic acid is a ligand for RAR, whereas 9-*cis* retinoic acid is a ligand for both RXR and RAR. RAR and RXR form heterodimers or homodimers and bind to specific sequences on DNA that are known as retinoic acid response elements (RARE) or retinoid-X response elements. Vitamin D_3 and thyroid hormone receptors may also form heterodimers with RXR. Many human genes are known to contain RARE, and this regulation of gene expression may explain the potent effects of vitamin A on immunity and other biologic functions. The long terminal repeat of retroviruses contains a RARE, and in vitro studies have suggested that all-*trans* retinoic acid may either increase or decrease HIV replication, depending on the cell line and culture conditions [13–16]. Although low serum or plasma vitamin A levels have been associated with adverse clinical outcome during HIV infection, the effect of high-dose vitamin A supplementation on circulating HIV load is unknown. We conducted a randomized, double-masked, placebo-controlled clinical trial of high-dose vitamin A supplementation for HIV-infected adults.

Materials and Methods

Study population. The study population consisted of HIV-seropositive injection drug users that were being followed in a longitudinal study of HIV infection (the AIDS Linked to Intravenous Experiences [ALIVE] study) in Baltimore. The ALIVE study participants were recruited through extensive community outreach [17], and the cohort includes 630 HIV-seropositive adults. Participants in the ALIVE study are seen every 6 months in a community-based study clinic; a history is taken and a physical examination, complete blood cell count, and T cell subset studies are done at each visit. A consecutive sample of adults from the ALIVE study was seen during their regular semiannual visits, and members were offered the opportunity to participate in the clinical trial of vitamin A supplementation.

Received 31 December 1996; revised 7 October 1997.

Written informed consent was obtained from all patients. Study protocol was approved by the Johns Hopkins School of Hygiene and Public Health Committee on Human Research.

Grant support: NIH (DA-04334, DA-05566, AI-35143, HD-30042, HD-32247); United States Agency for International Development (Cooperative Agreement DAN-0045-A-5094-00).

Reprints or correspondence: Dr. Richard D. Semba, Suite 700, 550 North Broadway, Baltimore, MD 21205.

The Journal of Infectious Diseases 1998;177:611–6
© 1998 by The University of Chicago. All rights reserved.
0022-1899/98/7703-0013$02.00

128

Study design. The study was a randomized, double-masked, placebo-controlled clinical trial of a single dose of oral vitamin A, 60-mg retinol equivalent (RE) (200,000 IU). The vitamin A dose of 60-mg RE was chosen because it is widely used in developing countries for periodic supplementation of preschool children and for single-dose supplementation for lactating women. There are no other existing recommendations for the use of high-dose vitamin A, 60 mg RE, in adults. Eligibility criteria included age ≥18 years, seropositivity for HIV by ELISA and Western blot, not currently taking >10,000 IU daily of vitamin A supplements, and a CD4 lymphocyte count of <500 cells/μL on the previous ALIVE study visit (6 months prior). Women were excluded if they were pregnant or had a positive urine pregnancy test for human chorionic gonadotropin. Subjects were requested not to take vitamin A supplements or other supplements containing vitamin A during the study.

The participants were asked about current and past use of any medications and vitamin supplements. A morbidity history of the last 6 months and the last 7 days, including symptoms, clinic visits, and hospitalizations, was obtained at the baseline visit. The subjects were sequentially given a clinical trial study number. Each study number was previously assigned to either vitamin or placebo allocation, using a random number table in blocks of 10. Each subject received an envelope, which was labeled on the outside with the study number, containing one capsule. The vitamin A and placebo capsules were identical in appearance, smell, and taste (Task Force Sight and Life, Basel, Switzerland). The subjects took the capsule directly under the observation of an investigator. A blood sample for measurement of plasma vitamin A level, T cell subsets, and HIV RNA virus load was obtained at baseline and 2 and 4 weeks after enrollment in the clinical trial.

Laboratory methods. Antibody to HIV type 1 was measured using an EIA (Genetic Systems, Seattle) with Western blot (DuPont NEN, Wilmington, DE) for confirmation. A complete blood cell count with differential was done using an automated cell counter (SmithKline, King of Prussia, PA). T cell subset studies were done by use of flow cytometry on fresh specimens as described [18]. Plasma retinol levels were measured using high-performance liquid chromatography as described elsewhere [6]. Vitamin A reference standards from the National Institute of Standards and Technology (Gaithersburg, MD) and pooled vitamin A reference standards were run with study samples to monitor accuracy. All plasma samples were stored at −70°C prior to testing. HIV-1 RNA virus load was measured using a commercially available bDNA kit, which has a quanitification limit of 10,000 copies/mL (Chiron, Emeryville, Ca).

Statistical analysis. The sample size of 60 per study arm was chosen on the basis of $(1-\beta) = .80$, $\alpha = .05$, and a two-sided test to detect a 30% change in CD4 lymphocyte count [11] given a known background mean CD4 lymphocyte count of 275 (SD = 160) in this subgroup of the ALIVE cohort. Basic descriptive statistics were performed on demographic and laboratory variables for the entire study population and for each treatment group, separately at each of the three visits. Preliminary group comparisons were done using Wilcoxon's rank sum test for continuous outcomes (e.g., CD4 lymphocyte counts) or Fisher's exact test for dichotomous outcomes (e.g., use of antiretroviral therapy—yes/no).

The preliminary comparisons were done at each visit, separately, with the understanding that in the setting of multiple comparisons an alpha level of $\alpha/3 = 0.0167$ should be used as the significance level. The group comparisons done at baseline were done to evaluate the effectiveness of randomization and to identify potential confounders for which to control in the final analysis. When comparing virus load levels between the treatment groups, linear regression models were used to adjust for potential confounders, such as baseline CD4 lymphocyte count and plasma vitamin A levels. Spearman's correlation coefficients were also calculated to assess the degree of association between two continuous variables (e.g., plasma vitamin A levels and virus load levels).

The effect of vitamin A therapy on plasma vitamin A levels was examined using a paired t test to compare plasma vitamin A levels before and after (2 and 4 weeks) treatment. An alpha level of $\alpha/2 = .025$ was used as a level of significance. Linear regression techniques were also applied to compare the posttreatment vitamin A levels of the treatment and control groups while controlling for baseline vitamin A levels. The vitamin A levels 2 weeks after baseline were compared to examine immediate differences, and the average of the vitamin A levels at 2 and 4 weeks were compared between the treatment groups to examine more sustained differences.

A mixed model [19] was used to determine whether vitamin A therapy alters HIV load, while controlling for time-dependent confounders, such as CD4 lymphocyte count and vitamin A levels and accounting for the correlation between repeated measures. The baseline HIV load was considered as a confounder, and the multivariate vector of outcomes consisted of the follow-up virus load. This implies that only one parameter was needed to model the within-subject correlation between the two follow-up HIV load measures, and the correlation coefficient was assumed to be common across all subjects. The normality of HIV load was improved after a log transformation. The generalized estimating equations (GEE) approach [20] was also used to test the robustness of this method in regard to the multivariate normality assumption, and the results were equivalent. Only the mixed model results are presented for clarity. The statistical software package SAS [21] was used for all analyses.

Results

Of 120 subjects enrolled in the clinical trial, 113 (94.2%) returned for the 2-week visit and 110 (91.7%) returned for the 4-week visit, with a similar follow-up rate in the 2 treatment groups. The demographic characteristics of the participants at baseline are presented in table 1. The subjects included 89 men, 117 blacks and 3 whites, and 53 subjects previously diagnosed as having AIDS [22]. The average age of the participants was 38.2 years. There were no significant baseline differences between treatment groups in regard to age, sex, plasma vitamin A level, HIV load, CD4 lymphocyte count, self-reported morbidity, or use of multivitamins. Two and 4 weeks after enrollment, there were no differences between the treatment groups in the use of antiretroviral therapy or other medications (data not shown). At the time of the study, zidovudine, dideoxyinosine, and dideoxycytidine were the only antiretroviral medications in use in this study population.

Table 1. Baseline characteristics of HIV-infected injection drug users in 2 treatment groups.

Characteristic	Treatment groups: Vitamin A (n = 60)	Treatment groups: Placebo (n = 60)	P*
Age (years)	38 (34–42)	38 (34.5–43)	.544
Sex (male/female)	43/17	46/13	.677
AIDS diagnosis	29 (48%)	24 (40%)	.462
Plasma vitamin A			
Mean (μmol/L)	1.61 (1.15–1.88)	1.37 (1.09–1.63)	.083
% <1.05 μmol/L	10 (19%)	12 (23%)	.811
HIV load (copies/mL)	13,192 (8233–25,089)	15,776 (10,576–29,538)	.141
Log HIV load†	9.49 (9.02–10.13)	9.67 (9.27–10.29)	.141
CD3 lymphocyte count (cells/μL)	1142 (834–1590)	1103 (768–1330)	.301
CD4 lymphocyte count (cells/μL)	296 (190–420)	259 (136–327)	.103
CD8 lymphocyte count (cells/μL)	786 (586–1114)	727 (550–1039)	.600
CD4%	18.5 (12–24.5)	17.5 (12–22.5)	.313
Body mass index (weight/height^2)	21.1 (20.0–23.4)	21.6 (19.3–24.0)	.933
Hemoglobin (g/L)	13.3 (12.4–15.5)	13.2 (11.6–14.3)	.231
Current medication use			
Anti-retroviral‡	30 (50%)	25 (43%)	.453
Multivitamins	18 (31%)	23 (38%)	.442

NOTE. Data are median (interquartile range), or n (%).
* Fisher's exact test for categorical variables or Wilcoxon rank sum test for continuous variables.
† Natural log of HIV load [$\log_e$(copies/mL)].
‡ Two subjects missing medication information at baseline.

Plasma vitamin A level in the 2 treatment groups at baseline and follow-up are shown in figure 1. As determined by use of a nonparametric t test, the plasma vitamin A levels were similar in the 2 groups at baseline and at the two follow-up visits. At visit two, the plasma vitamin A levels for the 2 treatment groups were compared, while controlling for the baseline vitamin A level using linear regression, and no treatment effect was noted (P = .06). However, when a similar linear regression model was applied on the average of vitamin A levels at visits two and three, while controlling for baseline vitamin A level, we found a significant treatment effect at follow-up (P = .01).

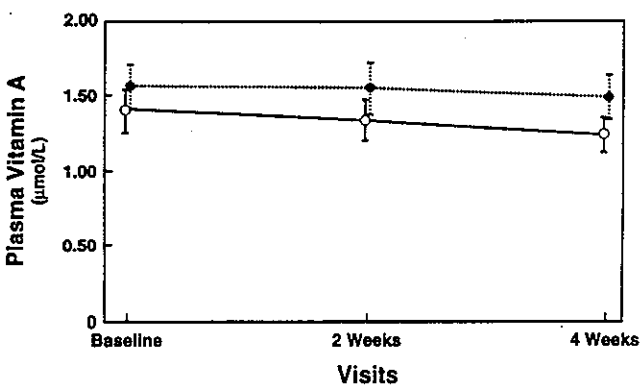


Figure 1. Mean plasma vitamin A levels in 2 treatment groups of HIV-infected injection drug users at baseline and follow-up. ●, vitamin A; ○, placebo. Bars indicate 95% confidence intervals.

In addition, when we compared the baseline visit to the follow-up visits for assessing a change in plasma vitamin A level in the treatment group, there was no difference at visit two (P = .88) or visit three relative to baseline (P = .10). In contrast, there was a decrease in plasma vitamin A levels of −.012 μmol/L from baseline to visit two (P = .04) and of −.013 μmol/L from visit two to visit three (P = .01) in the placebo group. The results suggest that the vitamin A level of the treatment group remained fairly stable from baseline to the follow-up visits, whereas those of the placebo group decreased. The linear slopes in vitamin A levels over the three visits also significantly declined in the placebo group (P = .01) but not in the treatment group (P = .07).

Circulating HIV load in the 2 treatment groups at baseline and follow-up is shown in figure 2. Log HIV load was similar for treatment groups at baseline (P = .14) and at the two follow-up visits (P = .17). We applied a linear regression model to assess the randomization with respect to HIV virus load at baseline, while controlling for baseline CD4 lymphocyte count and baseline vitamin A level. In the linear regression model, the baseline CD4 lymphocyte count was associated with baseline HIV load (P = .0001). There was a borderline relationship between baseline HIV load and baseline vitamin A level (P = .053) but no relationship to treatment allocation (P = .65).

CD4 lymphocyte counts in the 2 treatment groups at baseline and follow-up are shown in figure 3. There were no differences in CD4 lymphocyte count at baseline (P = .10), the second

130

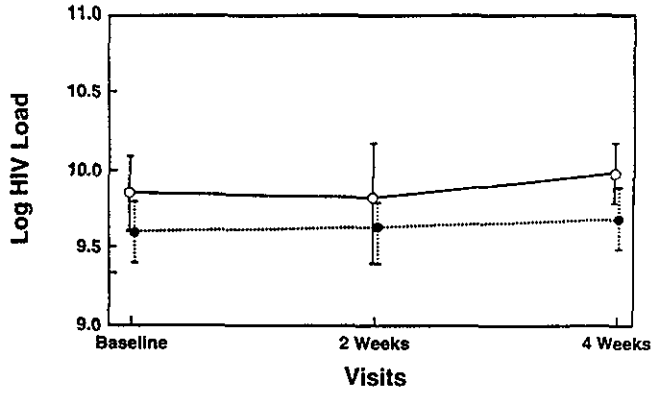


Figure 2. Mean natural log HIV RNA virus load [$\log_e$(copies/mL)] in 2 treatment groups of HIV-infected injection drug users at baseline and follow-up. ●, vitamin A; ○, placebo. Bars indicate 95% confidence intervals.

Table 2. Final linear regression model for effect of vitamin A supplementation on log HIV load.

Variable	Parameter estimate*	SE	*P*
Baseline plasma vitamin A (μmol/L)	−0.0040	0.0027	.1367
Baseline CD4 lymphocyte count (cells/μL)	0.3557×10^{-4}	0.0003	.9188
Baseline HIV load (log copies/mL)†	0.7693	0.0619	.0001
Current CD4 lymphocyte count (cells/μL)	-0.7604×10^{-3}	0.0003	.0309
Vitamin A supplement	0.0045	0.0862	.9584

NOTE. Mixed linear regression model was obtained via procedure mixed in SAS [21].
* Maximum likelihood parameter estimates.
† Natural log of HIV virus load [$\log_e$(copies/mL)].

visit (P = .76), or the third visit (P = .17) between the 2 groups. This clinical trial was conducted within the context of the ALIVE study, and information on AIDS diagnosis and T cell subsets was available both before and after the trial. Because of concern that vitamin A may not influence T cell subsets in subjects with failed T cell homeostasis and with AIDS [23], we excluded subjects who were diagnosed with AIDS either prior to baseline or within 2 years of baseline for the clinical trial; no effect of treatment on CD4 lymphocyte count was noted (data not shown). In addition, vitamin A supplementation did not have a significant effect on CD3, CD4, or CD8 lymphocyte counts at the next ALIVE study visit 6–9 months later (data not shown).

At baseline, hemoglobin levels were similar between the 2 treatment groups. There were no significant changes in hemoglobin levels in either group from baseline to the follow-up visits. It has been suggested that plasma vitamin A levels may be correlated with CD4 lymphocyte counts and hemoglobin levels, given the role that vitamin A plays in both lymphopoiesis and hematopoiesis [4, 5]. No significant correlations were noted between plasma vitamin A level and CD4 lymphocyte count at any of the visits (P > .15). We estimated negative correlations between plasma vitamin A level and HIV load at each visit; however, no correlation estimates were significant. The strongest correlation coefficient was estimated at visit one (ρ = −0.19, P = .058). Plasma vitamin A levels were positively correlated with hemoglobin levels at each of the three visits, ranging in estimate from 0.33 to 0.42 (P < .001).

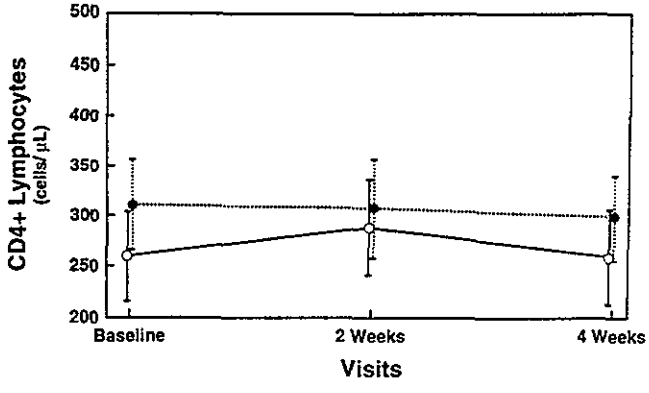


Figure 3. Mean CD4 lymphocyte count in 2 treatment groups of HIV-infected injection drug users at baseline and follow-up. ● vitamin A; ○, placebo. Bars indicate 95% confidence intervals.

The final mixed model shown in table 2 presents the effect of treatment, baseline CD4 lymphocyte count, baseline plasma vitamin A level, baseline HIV load, and the time-dependent CD4 lymphocyte count on HIV load. It appears that only baseline HIV load and current time-dependent CD4 lymphocyte count influence the level of HIV load. The treatment effect was not significant (β =.004, P = .96). When subjects with a prior AIDS diagnosis were excluded, the mixed model results in table 2 did not change.

Because most subjects in this trial had vitamin A levels in the normal range at baseline, an analysis was done to determine whether a treatment effect occurred among those who had low vitamin A levels. Comparisons within the group of 22 subjects who at baseline had vitamin A levels consistent with deficiency (plasma vitamin A <1.05 μmol/L) showed no significant increase in plasma vitamin A level after supplementation. A linear regression analysis using the GEE model showed no treatment effect on HIV load while controlling for virus load, vitamin A level, and CD4 lymphocyte count at baseline.

Discussion

The present study is limited in that only 22 of the HIV-infected adults in this study had plasma vitamin A levels at baseline that were consistent with deficiency. Thus, high-dose vitamin A supplementation was used in a population in which only a quarter of the subjects had low plasma vitamin A levels.

The decreased plasma vitamin A levels in this population of injection drug users may reflect an inadequate intake of vitamin A–containing foods [24], fat malabsorption (which lowers absorption of fat-soluble vitamins) [25], and abnormal urinary losses of vitamin A during HIV infection [26]. Circulating vitamin A levels may be subject to autoregulation in individuals who have adequate liver vitamin A stores [27], and this may explain why plasma vitamin A levels did not increase in the treatment group at 2 and 4 weeks following supplementation. Other factors that may limit the release of vitamin A from the liver include zinc deficiency and protein energy malnutrition; however, body mass index, an indicator of protein energy status, was >20, or in the "ideal" range [28] for >75% of the subjects. The study suggests, however, that vitamin A supplementation provided some protection against a decline in plasma vitamin A levels, which has been described during HIV infection [6].

This study suggests that one high dose of 60-mg RE vitamin A orally does not lower virus load levels in adults, and a stratified analysis suggests that there was no influence on HIV load among those with vitamin A levels <1.05 μmol/L. The single dose of 60-mg RE vitamin A may have been insufficient to have an effect on virus load or CD4 lymphocyte count. Two doses of 60-mg RE vitamin A on consecutive days are recommended to treat acute measles in children [29], and a single dose of 90-mg RE vitamin A has been used in lactating women [30]. It is unknown whether similar, higher single doses or multiple doses of vitamin A might have an effect on virus load or CD4 lymphocyte counts. It is possible that there may have been a change in virus load immediately after dosing with vitamin A; however, this study did not measure virus load within the first few days of dosing.

During HIV infection, vitamin A levels <1.05 μmol/L have been associated with lower CD4 cell counts [31, 32]. Possible explanations for the association between low CD4 lymphocyte counts and low vitamin A levels may be the poorer nutritional status that sometimes accompanies more advanced HIV disease, the impairment of lymphopoiesis by lack of vitamin A, or both. However, we did not see a significant direct correlation between plasma vitamin A and CD4 lymphocyte counts in the present study. A clinical trial conducted among HIV-infected children suggests that vitamin A supplementation increases circulating NK and CD4 lymphocyte counts dramatically following two daily sequential doses of 60-mg RE vitamin A [11].

Low vitamin A levels in HIV-infected women have been associated with higher HIV load in breast milk [9], which might partially explain the association between low maternal vitamin A levels and increased mother-to-child transmission of HIV [8]. It has been suggested that vitamin A supplementation of lactating women may reduce HIV load in breast milk and thus mother-to-child transmission of HIV. The present study shows that a single high-dose vitamin A supplementation has no effect on circulating HIV load 2 and 4 weeks after dosing. There are other biologic mechanisms by which vitamin A supplementation may potentially reduce mother-to-child transmission of HIV, such as by reducing chorioamnionitis during late pregnancy or by supplying the fetus with sufficient vitamin A for normal immune development. Four clinical trials involving >3000 HIV-infected pregnant women are currently in progress to determine whether antenatal vitamin A supplementation can reduce mother-to-child transmission of HIV [33]. The present study suggests vitamin A is unlikely to increase or decrease HIV load, but other potential mechanisms still exist by which vitamin A could have a beneficial effect in these trials.

References

1. Liang B, Chung S, Araghiniknam M, Lane L, Watson RR. Vitamins and immunomodulation in AIDS. Nutrition 1996;12:1–7.
2. Kotler DP, Grunfeld C. Pathophysiology and treatment of the AIDS wasting syndrome. In: Volberding P, Jacobson MA, eds. AIDS clinical review 1995/1996. New York: Marcel Dekker, 1995:229–75.
3. Tang AM, Graham NMH, Kirby AJ, McCall LD, Willett WC, Saah AJ. Dietary micronutrient intake and risk of progression to acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus type 1 (HIV-1)–infected homosexual men. Am J Epidemiol 1993;138: 937–51.
4. Ross AC. The relationship between immunocompetence and vitamin A status. In: Sommer A, West KP Jr. Vitamin A deficiency: health, survival, and vision. New York: Oxford University Press, 1996:251–76.
5. Semba RD. Vitamin A, immunity, and infection. Clin Infect Dis 1994;19: 489–99.
6. Semba RD, Caiaffa WT, Graham NMH, Cohn S, Vlahov D. Vitamin A deficiency and wasting as predictors of mortality in human immunodeficiency virus–infected injection drug users. J Infect Dis 1995;171: 1196–202.
7. Rwangabwoba JM, Humphrey J, Coberly J, Moulton L, Desormeaux J, Halsey NA. Vitamin A status and development of tuberculosis and/or mortality [abstract We.B.3268]. In: Program and abstracts: XI International Conference on AIDS (Vancouver, Canada). Vol 2. Vancouver: XI International Conference on AIDS Society, 1996:103.
8. Semba RD, Miotti PG, Chiphangwi JD, et al. Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. Lancet 1994;343: 1593–7.
9. Nduati RW, John GC, Richardson BA, et al. Human immunodeficiency virus type 1–infected cells in breast milk: association with immunosuppression and vitamin A deficiency. J Infect Dis 1995;172:1461–8.
10. Coutsoudis A, Bobat RA, Coovadia HM, Kuhn L, Tsai WY, Stein AZ. The effects of vitamin A supplementation on the morbidity of children born to HIV-infected women. Am J Public Health 1995;85:1076–81.
11. Hussey G, Hughes J, Potgieter S, et al. Vitamin A status and supplementation and its effects on immunity in children with AIDS [abstract]. In: Abstracts of the XVII International Vitamin A Consultative Group Meeting (Guatemala City). Washington, DC: International Life Science Institute, Human Nutrition Institute, 1996:6.
12. De Luca LM. Retinoids and their receptors in differentiation, embryogenesis, and neoplasia. FASEB J 1991;5:2924–33.
13. Collins SJ. Retinoic acid–induced differentiation of retrovirus-infected HL-60 cells is associated with enhanced transcription from the viral long terminal repeat. J Virol 1988;62:4349–52.
14. Orchard K, Lang G, Harris J, Collins M, Latchman D. A palindromic element in the human immunodeficiency virus long terminal repeat binds retinoic acid receptors and can confer retinoic acid responsiveness on a heterologous promotor. J Acquir Immune Defic Syndr 1993;6: 440–5.

132

15. Poli G, Kinter AL, Justement JS, Bressler P, Kehrl JH, Fauci AS. Retinoic acid mimics transforming growth factor β in the regulation of human immunodeficiency virus expression in monocytic cells. Proc Natl Acad Sci USA 1992;89:2689–93.
16. Turpin JA, Vargo M, Meltzer MS. Enhanced HIV-1 replication in retinoid-treated monocytes: retinoid effects mediated through mechanisms related to cell differentiation and to a direct transcriptional action on viral gene expression. J Immunol 1992;148:2539–46.
17. Vlahov D, Anthony JC, Muñoz A, et al. The ALIVE study, a longitudinal study of HIV-1 infection in intravenous drug users: description of methods and characteristics of participants. NIDA Res Monogr 1991;109: 75–100.
18. Giorgi JV, Cheng HL, Margolick JB, et al. Quality control in the flow cytometric measurement of T-lymphocyte subsets: the Multicenter AIDS Cohort Study Experience. Clin Immunol Immunopathol 1990; 55:173–86.
19. Wolfinger R. A tutorial on mixed models. Cary, NC: SAS Institute, 1992.
20. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986;73:13–22.
21. SAS Institute. SAS Institute SAS/STAT software: changes and enhancements through release 6.11. Cary, NC: SAS Institute, 1996.
22. Centers for Disease Control. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR 1987;36(suppl 1):1S–15S.
23. Margolick JB, Muñoz A, Donnenberg AD, et al. Failure of T-cell homeostasis preceding AIDS in HIV-1 infection. Nat Med 1995;1:674–80.
24. Smit E, Graham NMH, Tang A, Flynn C, Solomon L, Vlahov D. Dietary intake of community-based HIV-1 seropositive and seronegative injecting drug users. Nutrition 1996;12:496–501.
25. Koch J, Garcia-Shelton YL, Neal EA, et al. Steatorrhea: a common manifestation in patients with HIV/AIDS. Nutrition 1996;12:507–10.
26. Jolly PE, Yang YL, Alvarez JO, Smoot TM. Vitamin A depletion in HIV infection and AIDS [letter]. AIDS 1996;10:114.
27. Olson JA. Biochemistry of vitamin A and carotenoids. In: Sommer A, West KP Jr, eds. Vitamin A deficiency: health, survival, and vision. New York: Oxford University Press, 1996:221–50.
28. Gibson RS. Principles of nutritional assessment. New York, Oxford University Press, 1990.
29. Sommer A, West KP Jr, eds. Vitamin A deficiency: health, survival, and vision. New York: Oxford University Press, 1996.
30. Stoltzfus RJ, Hakimi M, Miller KW, et al. High-dose vitamin A supplementation of breast feeding Indonesian mothers: effects on the vitamin A status of mother and infant. J Nutr 1993;123:666–75.
31. Phuapradit W, Chaturachinda K, Tannepanichskul S, Sirivarasry J, Khupulsup K, Lerdvuthisopon N. Serum vitamin A and beta-carotene levels in pregnant women infected with human immunodeficiency virus–1. Obstet Gynecol 1996;87:564–7.
32. Semba RD, Park S, Royal W, Griffin DE. Vitamin A deficiency and T-cell subpopulations in HIV-infected adults. Nutr Res 1996;16:915–23.
33. Semba RD. Overview of the potential role of vitamin A in mother-to-child transmission of HIV-1. Acta Paediatr Suppl 1997;421:107–12.

133

Table 1. HIV seroprevalence among women of reproductive age.

	Total	HIV-infected (%)	OR (95% CI)
Parturients	5338	2.3	1.0
Women with ectopic pregnancy	151	2.6	1.15 (0.36–3.29)
Women undergoing elective abortion	453	1.1	0.47 (0.17–1.21)
Women with spontaneous abortion	143	2.1	0.91 (0.23–2.99)
PID inpatients	349	7.7	3.56 (2.25–5.57)
PID outpatients	119	4.2	1.86 (0.66–4.82)

OR, Odds ratio; CI, confidence interval; PID, pelvic inflammatory disease.

for differences in age or ethnicity using data abstracted from medical records, results were not affected for the groups examined.

Although limited by small sample size, these data illustrate several points. Women with PID were more likely than parturients to be HIV-positive (statistically significant for inpatients only). This finding supports the recommendation of the Centers for Disease Control and Prevention that women with PID be offered HIV counseling and testing [5]. HIV seroprevalence among women with ectopic pregnancy, elective or spontaneous abortions was very similar to seroprevalence among parturients. The comparability of observed seroprevalence rates among all women who conceived suggests that data from surveys of parturients may be an appropriate surrogate for HIV prevalence among most women of reproductive age from the same population. However, extrapolation from surveys of parturients may underestimate rates in some populations, for example, those with large numbers of 'high-risk' women, such as PID patients.

A.C. Duerr, S.V. Brockmann*, C.T. Cesnik-Barbosa and M.A. Macasaet†, *Centers for Disease Control and Prevention, Atlanta, GA 30341, *Department of Community Medicine, and †Department of Community Medicine, State University of New York at Brooklyn, New York, USA.*

Date of receipt: 23 September 1997; accepted: 25 September 1997.

References

1. *The Status and Trends of the Global HIV/AIDS Pandemic Symposium Final Report, Vancouver, July 1996.* Vancouver: AIDS Control and Prevention (AIDSCAP) Project/Family Health International, Harvard School of Public Health and The Joint United Nations Programme on HIV/AIDS; 1996.
2. Couturier E, Brossard Y, Larsen C, *et al.*: **HIV infection at outcome of pregnancy in the Paris area, France.** *Lancet* 1992, **340**:707–709.
3. Obadia Y, Rey D, Moatti JP, *et al.*: **HIV prenatal screening in south-east France: differences in seroprevalence and screening policies by pregnancy outcome.** *AIDS Care* 1994, **6**:29–38.
4. Barbosa C, Macasaet M, Brockmann S, Sierra M, Xia Z, Duerr A: **Pelvic inflammatory disease and human immunodeficiency virus infection.** *Obstet Gynecol* 1997, **89**:65–70.
5. Centers for Disease Control: **Pelvic inflammatory disease guidelines for prevention and management.** *MMWR* 1991, **40** (RR-5):11–25.

Maternal serum lactoferrin and vertical transmission of HIV

Lactoferrin is an iron-binding glycoprotein which is an important component of non-specific immunity to bacteria, fungi and viruses [1]. Human milk, blood, sweat, tears and gastrointestinal secretions contain lactoferrin, which is produced by both exocrine glands and neutrophils. Lactoferrin is a potent inhibitor of HIV replication *in vitro* [1], and circulating lactoferrin levels are decreased in HIV-infected adults [2]. Although there are many studies to suggest that lactoferrin has potent immunological effects against bacteria and viruses *in vitro* [2,3], the clinical significance of circulating lactoferrin levels is unclear.

We examined the relationship between maternal serum lactoferrin levels and vertical transmission of HIV in a non-concurrent, prospective cohort study of 567 HIV-infected mothers and their infants in Malawi. Maternal age, body mass index, CD4 cell percentage, and serum lactoferrin levels were measured during the second or third trimester of pregnancy. Infant HIV status was determined at 12 months of age. Details of this cohort have been described elsewhere [4]. Briefly, human serum lactoferrin levels were measured by a sandwich enzyme-linked immunosorbent assay using plates coated with rabbit anti-human lactoferrin (Organon Teknika, Durham, North Carolina, USA), dilutions of human sera, peroxidase-conjugated rabbit immunoglobulin G to human lactoferrin (Organon Teknika), and substrate development with o-phenylenediamine dihydrochloride. Absorbances were read at 490 nm with wavelength correction at 630 nm. Purified human lactoferrin (Organon Teknika) was used as a standard to estimate the amount of serum lactoferrin.

Of 567 HIV-infected mothers, an adequate serum sample for serum lactoferrin measurement was available in 440 mothers (263 mothers who did not transmit HIV

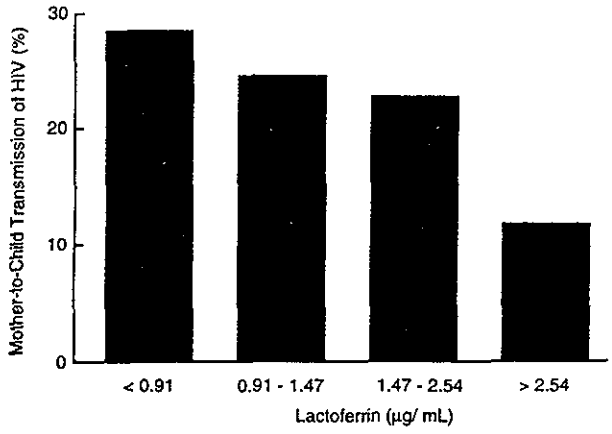


Fig. 1. Vertical transmission of HIV by quartile of maternal serum lactoferrin level (Mantel–Haenzsel $\chi^2 = 0.019$).

to their infants, 74 mothers who transmitted HIV to their infants, and 103 mothers whose infants died before 12 months). Amongst all the women, mean serum lactoferrin level was 1.98 µg/ml. Mean serum lactoferrin level was 1.50 ± 0.13 µg/ml in mothers with HIV-infected infants and 2.01 ± 0.09 µg/ml in mothers with uninfected infants ($P < 0.001$). From lowest to highest quartile of lactoferrin level, vertical transmission rates of HIV were 28.6, 24.7, 22.9 and 12.1%, respectively ($P = 0.019$; Fig. 1).

In order to compare the effects of maternal serum lactoferrin, body mass index, age, and CD4 cell percentage as cofactors for vertical transmission of HIV, we fit a multivariate logistic regression model in which the population SD for each variable was used as the unit of change. In this multivariate logistic regression model, maternal age (per 5.6 year increase), body mass index (per 3.0 kg/m^2 increase), CD4 cell percentage (per 10.9% increase), and serum lactoferrin (per 1.56 µg/ml increase) had odds ratios of 1.20 [95% confidence intervals (CI), 0.77–1.85], 1.07 (95% CI, 0.71–1.61), 0.58 (95% CI, 0.33–1.02), and 0.51 (95% CI, 0.26–1.01), respectively, for vertical transmission of HIV.

This study suggests that mean serum lactoferrin levels in HIV-infected pregnant women in Malawi are about 2 µg/ml, which is one-third to one-half lower than the mean lactoferrin levels of 3–4 µg/ml that have has been reported in healthy adults in industrialized countries [2,5]. Serum lactoferrin is largely derived from granulocytes, but neutropenia does not necessarily correlate well with circulating lactoferrin levels [2]. In HIV-negative individuals, elevated circulating lactoferrin levels are found during bacterial infections [5,6], and plasma lactoferrin levels and other markers of neutrophil activation have been reported to increase during pregnancy [7]. In contrast, lower levels of circulating lactoferrin have been described in symptomatic HIV-infected adults compared with asymptomatic adults and HIV-negative controls [2].

Low levels of circulating lactoferrin appear to be associated with increased mother-to-child transmission of HIV in this population. *In vitro* studies suggest that lactoferrin is a potent inhibitor of HIV replication [1], and deficiency of lactoferrin production in humans is associated with recurrent bacterial infections [8]. The granules of neutrophilic granulocytes contain high concentrations of lactoferrin, and it has been hypothesized that neutrophil activation and increased circulating lactoferrin levels comprise a normal immune defense mechanism during a healthy pregnancy [7]. It is unclear why serum lactoferrin levels are low in this population of HIV-infected pregnant women, but possible explanations include bone-marrow suppression or autoimmune dysfunction in HIV infection. Anti-lactoferrin antibodies have been reported to occur in over 60% of HIV-infected adults, and these antibodies may trap lactoferrin in circulating immune complexes and prevent its biological activity [2,9]. Further studies are needed to confirm these findings and to elucidate the potential role of circulating lactoferrin in HIV infection.

R.D. Semba, P.G. Miotti*, Y. Lan, J.D. Chiphangwi†, D.R. Hoover*, G.A. Dallabetta*, L-P. Yang* and A.J. Saah*, *Department of Ophthalmology, School of Medicine, *Department of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland, USA, and †College of Medicine, University of Malawi, Blantyre, Malawi.*

Date of receipt: 23 September 1997; accepted: 25 September 1997.

References

1. Harmsen MC, Swart PJ, de Béthune MP, *et al.*: **Antiviral effects of plasma and milk proteins lactoferrin show potent activity against both human immunodeficiency virus and human cytomegalovirus replication *in vitro*.** *J Infect Dis* 1995, **172**:380–388.
2. Defer MC, Dugas B, Picard O, Damais C: **Impairment of circulating lactoferrin in HIV-1 infection.** *Cell Mol Biol* 1995, **41**:417–421.
3. Lönnerdal B, Iyer S: **Lactoferrin: molecular structure and biological function.** *Annu Rev Nutr* 1995, **15**:93–110.
4. Semba RD, Miotti PG, Chiphangwi JD, *et al.*: **Maternal vitamin A deficiency and mother-to-child transmission of HIV-1.** *Lancet* 1994, **343**:1593–1597.
5. Bezwoda WR, Baynes RD, Khan Q, Mansoor N: **Enzyme linked immunosorbent assay for lactoferrin. Plasma and tissue measurements.** *Clin Chim Acta* 1985, **151**:61–69.
6. Scott PH: **Plasma lactoferrin levels in newborn preterm infants: effect of infection.** *Ann Clin Biochem* 1989, **26**:412–415.
7. Rebelo I, Carvalho-Guerra F, Pereira-Leite L, Quintanilha A: **Lactoferrin as a sensitive blood marker of neutrophil activation in normal pregnancies.** *Eur J Obstet Gynecol* 1995, **62**:189–194.
8. Boxer LA, Coates TD, Haak RA, Wolach JB, Hoffstein S, Baelmer RL: **Lactoferrin deficiency associated with altered granulocyte function.** *N Engl J Med* 1982, **307**:404–410.
9. Defer MC, Follézou JY, Picard O, Damais C: **Antilactoferrin antibodies associated with HIV infection.** *Comptes Rend Acad Sci* 1994, **317**:675–678.

135

VITAMIN A AND MALARIA

Association Between Measures of Vitamin A and the Ocular Fundus Findings in Cerebral Malaria

Susan Lewallen, MD; Terrie E. Taylor, DO; Malcolm E. Molyneux, FRCP; Richard D. Semba, MD; Bridget A. Wills, MRCP; Paul Courtright, DrPH

Objective: To investigate the relationship between serum vitamin A levels and conjunctival impression cytology and retinal whitening present in Malawian children with cerebral malaria.

Methods: Standard retinal examination and conjunctival impression cytology were performed at hospital admission on 101 consecutively admitted children with cerebral malaria. Blood samples were drawn from 56 children at 24 hours, frozen at −20°C, and transported for assessment of vitamin A levels by high-performance liquid chromatography. Associations among fundus findings and vitamin A measurements were sought.

Results: The whitening of the retina that we have previously described in children with cerebral malaria was found to be associated with a mean±SD serum vitamin A level of 0.29±0.1 μmol/L, compared with a mean vitamin A level of 0.41±0.2 μmol/L in children without retinal whitening. Children with retinal whitening were 2.77 (95% CI, 1.06-7.3) times more likely to have abnormal conjunctival impression cytology results than those without whitening. No child had any clinical or ophthalmologic evidence of chronic vitamin A deficiency.

Conclusions: The retinal whitening described in children with cerebral malaria is associated with low serum vitamin A levels and with abnormal conjunctival impression cytology results and may be due to acute vitamin A deficiency at the tissue level.

Arch Ophthalmol. 1998;116:293-296

From the British Columbia Centre for Epidemiologic and International Ophthalmology, Vancouver (Drs Lewallen and Courtright); International Eye Foundation (Drs Lewallen and Courtright), Malaria Project and Wellcome Trust Centre, University of Malawi College of Medicine (Drs Molyneux and Wills), Blantyre; College of Osteopathic Medicine, Michigan State University, East Lansing (Dr Taylor); Liverpool School of Tropical Medicine, Liverpool, England (Dr Molyneux); and the Wilmer Eye Institute, The Johns Hopkins University School of Medicine, Baltimore, Md (Dr Semba).

MORE THAN 500 million people live in areas where malaria is endemic.[1] Cerebral malaria is one of the most severe syndromes of infection by *Plasmodium falciparum*. In sub-Saharan Africa, malaria is responsible for death in 1 in 20 children younger than 5 years.[2] We have described changes in the appearance of the ocular fundus in children with cerebral malaria,[3] these changes being predictive of outcome.[4] One of the most distinctive of the features was a mosaic pattern of whitening of the retina; this occurs either in the macula or outside the arcades, in the midperipheral retina. Children with extramacular whitening had a relative risk of poor outcome (death or neurologic sequelae) 2.2 times (95% CI, 1.2-3.8) higher than those without this finding.

Occasionally the whitening is severe and coalesces into large areas (**Figure 1**). In these cases, the ophthalmologic appearance bears a striking resemblance to commotio retinae. The appearance of commotio retinae is due to trauma-induced abnormalities in the outer segment of the rods and cones.[5] In animal models, the outer segments of the rods are known to show degeneration in vitamin A deficiency.[6]

In the past few decades, the important role of vitamin A in host defense against infection has become increasingly well recognized. Vitamin A deficiency, even subclinical deficiency, has been shown to cause reduced childhood survival and to increase the incidence and severity of several infectious diseases; specifically, measles, diarrheal disease, urinary tract infections, otitis media, and possibly respiratory disease.[7] One of the difficulties in studying vitamin A is that there is no one best measure of vitamin A status. The various techniques available for assessing vitamin A, along with their limitations, are well described.[7] Serum vitamin A levels do not necessarily reflect total body stores of vitamin A and they may drop precipitously in the face of febrile illness; specifically, they have been shown to fall to very low levels during malaria attacks, even in subjects who are not likely to be vitamin A deficient.[8] Compared with serum vitamin A measurements, the conjunctival impression cytology technique may be a better measure of target tissue sufficiency of vitamin A. However, while it has been shown to be useful in measur-

137

PATIENTS AND METHODS

The patients in this study were a consecutive subset consisting of two thirds of the patients enrolled in the Malaria Research Project at the Queen Elizabeth Central Hospital in Blantyre during 1992 and 1993, as part of a multicenter trial comparing artemeter with quinine in the treatment of cerebral malaria. Inclusion criteria and clinical assessments have been described elsewhere.[4] Permission for this study was granted by the Health Science Research Committee of Malawi and children were enrolled only after informed consent was given by the child's guardian. Briefly, children were admitted if they had *P falciparum* malaria on blood smear, had a coma score of at least 2 on the Blantyre Coma Scale, and had no other cause of illness identified on clinical or laboratory assessment, which included culture of blood and cerebrospinal fluid. The examination on hospital admission included examination of the external eye by penlight and funduscopic inspection (after pupillary dilation) by indirect ophthalmoscopy by one ophthalmologist (S.L.); results were recorded on a standard form. Conjunctival impression cytology was performed on hospital admission according to previously described methods.[11] Briefly, vacuum pump–held precut filter paper circles were applied to the temporal conjunctiva of each eye, placed immediately in fixative and stained with periodic acid–Schiff reagent and Harris hematoxylin, then read in a masked fashion by 2 trained observers (S.L. and P.C.) and graded (based on the presence of goblet cells) as normal, abnormal, or unreadable. Problems with purity of reagents necessitated restaining of a number of conjunctival impression cytology samples. Plasma samples were collected at 24 hours after hospital admission and frozen at −20°C. Samples were transported after several months via liquid nitrogen dry shipper to Baltimore, Md, where one investigator (R.S.) measured vitamin A in a masked fashion using high-performance liquid chromatography.[12] Vitamin A reference standards from the National Institute of Standards and Technology, Gaithersburg, Md, and pooled vitamin A reference standards were run with the study samples to monitor accuracy.

ing the vitamin A status of populations,[9] its usefulness in individual patients has been questioned.[10]

We studied both serum vitamin A levels and conjunctival impression cytology results in the context of detailed clinical and ophthalmologic assessments in children with cerebral malaria enrolled in the Malaria Research Project in Blantyre, Malawi, in 1993 and 1994, and present an analysis of the findings.

RESULTS

We performed retinal examinations and conjunctival impression cytology on 101 children. Fifty-six of these were consecutive admissions and had serum samples available for vitamin A measurement.

Figure 1. *A severe case showing midperipheral whitening that is confluent in some areas, resembling commotio retinae.*

Association Between Presence of Retinal Whitening and Vitamin A Status

	Serum Vitamin A Level, µmol/L (Mean±SD)	Conjunctival Impression Cytology Results	
		Abnormal	Normal
Retinal whitening*			
Present	0.29±0.11 (n=20)	17	18
Absent	0.41±0.16 (n=36)†	16	47
Papilloedema‡			
Present	0.41±0.15 (n=7)	4	6
Absent	0.36±0.15 (n=49)	29	59

**Odds ratio=2.77 (95% CI, 1.06-7.3), P<.05.*
†P<.01.
‡Odds ratio=1.36 (95% CI, 0.29-6.04).

Thirty-five (35%) of the children had retinal whitening. In 15 the whitening was confined to the macula, in 3 it was extramacular only, and in 17 there was both macular and extramacular whitening.

Serum vitamin A concentrations ranged from 0.06 to 0.84 µmol/L, with a mean±SD of 0.37±0.15 µmol/L and a median of 0.36 µmol/L. Conjunctival impression cytology results were normal in 65 children, abnormal in 33, and unreadable in 3.

Patients with retinal whitening had a mean±SD serum vitamin A concentration of 0.29±0.11 µmol/L, while children without retinal whitening had a mean±SD serum vitamin A level of 0.41±0.16 µmol/L (P=.002) (**Table**). Children with retinal whitening were 2.77 (95% CI, 1.06-7.3) times as likely to have an abnormal conjunctival impression cytology result as children without retinal whitening. There was no association between serum vitamin A levels or conjunctival impression cytology results and papilloedema or retinal hemorrhages.

The mean±SD serum vitamin A level in children with abnormal conjunctival impression cytology results was 0.34±0.14 µmol/L compared with 0.40±0.16 µmol/L in

138

children with a normal conjunctival impression cytology result (nonsignificant).

There was no association between outcome (death) and serum vitamin A concentration. Nine (27%) children with abnormal conjunctival impression cytology results and 13 children (20%) with normal conjunctival impression cytology results died (nonsignificant).

COMMENT

We have demonstrated an association between 2 different measures of vitamin A status and the retinal whitening that occurs in children with cerebral malaria. A possible explanation for this association is that the retinal whitening is a consequence of low serum vitamin A levels or reduced vitamin A availability at the tissue level. The retinopathy of vitamin A deficiency in humans has been described as consisting of white or yellow lesions, deep in the retina, patchy or punctate, in the midperiphery, and most pronounced along vessels. The typical whitening we see in cerebral malaria is indeed a patchy mosaic (though not punctate), it is frequently most prominent along vessels, and it is deep in the retina (**Figure 2**). It is often present in the midperiphery but may also or exclusively involve the macula. This last characteristic differs from reports of vitamin A deficiency retinopathy, which is described as always occurring outside the temporal vascular arcade.[7,13] We have noted that when the macula is affected in cerebral malaria, the foveola is always completely spared (**Figure 3**). This could be due to the fact that the foveola is predominantly cones, which do not have the same dependence on vitamin A as do the rods. These considerations, along with our epidemiologic evidence, lend further support to the possibility that the whitening may be a manifestation of vitamin A deficiency at the tissue level. It is not clear, however, whether these children actually have systemic vitamin A deficiency, because the acute-phase reaction itself causes an acute lowering of serum vitamin A concentration.

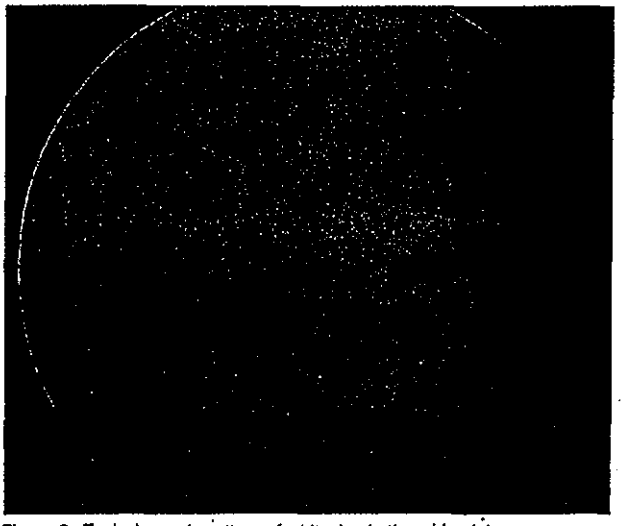


Figure 2. *Typical mosaic pattern of whitening in the midperiphery.*

Reported descriptions of retinopathy in vitamin A deficiency are from patients who have decreased vitamin A stores, either due to limited dietary intake of vitamin A or to disease that leads to limited absorption of ingested vitamin A. These patients presumably are in a true state of chronic vitamin A deficiency. None of the children in our study exhibited the macroscopic conjunctival or corneal manifestations of chronic vitamin A deficiency. Some (those with abnormal conjunctival impression cytology results) may well have had mild or moderate vitamin A deficiency, but others (those with normal conjunctival impression cytology results) are likely to have had sufficient vitamin A stores. Acute lowering of serum vitamin A concentration is a feature of the acute-phase response[8] occurring in the children in our study, all of whom had low serum vitamin A levels. Children with both normal and abnormal conjunctival impression cytology results showed retinal whitening, although the retinal lesions were more frequent among those

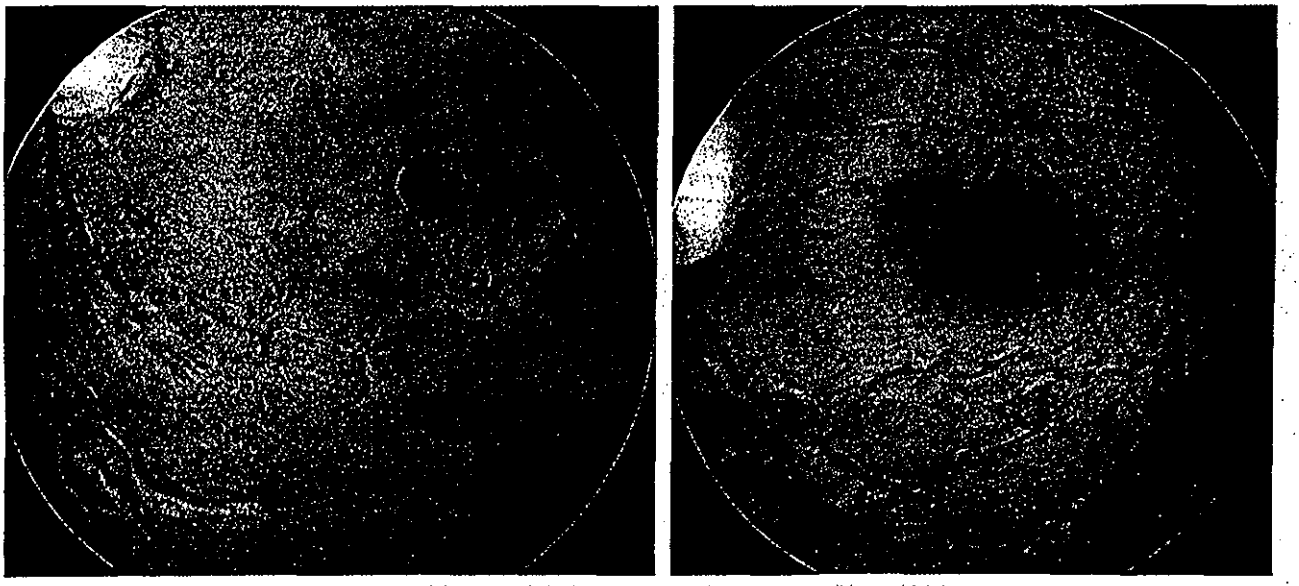


Figure 3. *Whitening in the macula. Note the sparing of the foveola in both a florid case (left) and a more mild case (right).*

139

with abnormal conjunctival impression cytology results. A possible interpretation of these findings is that an acute drop in serum vitamin A levels, such as occurs in the acute-phase response in malaria, is enough to cause the retina to show the signs resembling those of vitamin A deficiency, especially in children with already impaired tissue vitamin A status (those with abnormal conjunctival impression cytology results).

We have followed up several children who manifested retinal whitening during the episode of cerebral malaria and have found that the whitening disappears during a period of weeks to months, leaving no sequelae detectable on funduscopy. The children's diets are not supplemented with vitamin A during or after the malaria episode. If acute vitamin A deprivation causes the retinal lesions, then the rise in serum vitamin A levels associated with the cessation of the acute-phase response is enough to allow the retina to recover.

Other recent reports and photographs of fundus findings similar to ours come from Kenyan children with severe malaria. Researchers there found the same pattern of whitening and demonstrated by fluorescein angiography that this is not associated with leakage of vessels.[14]

We have examined many children in the same setting with coma and fever from other causes, such as encephalitis, meningitis, and poisoning, who are also undergoing an acute-phase reaction, and have never seen this pattern of whitening except in cases of malaria. We have seen it predominantly in cerebral malaria as opposed to less severe malaria[15] and the retinal whitening is only one component of the spectrum of retinal changes in children with cerebral malaria.[3] It therefore seems likely that additional mechanisms contribute to retinal abnormalities in severe malaria.

Our findings raise many questions about the possible role of altered vitamin A metabolism or availability in the pathophysiology and prognosis of cerebral malaria. Nevertheless, we hope that the identification of inverse correlations between retinal whitening and the levels of tissue and serum vitamin A will encourage further investigations into a phenomenon with potentially important therapeutic implications.

Accepted for publication November 26, 1997.

Funding for the vitamin A and conjunctival impression cytology measurements was provided by the International Eye Foundation, Bethesda, Md, the Hoffmann-LaRoche Sight and Life Programme, Basel, Switzerland, and the US Agency for International Development, Washington, DC (Cooperative Agreement DAN-0045-A-5094-00). Financial support for the care of children with severe and complicated malaria was provided by a grant from the Product Development Unit of the World Health Organization, Geneva, Switzerland.

We thank the patients, their parents, and the staff of the Malaria Research Project, the Department of Paediatrics of the College of Medicine, University of Malawi, and the Senior Medical Superintendent of the Queen Elizabeth Central Hospital, Blantyre (Ministry of Health and Population, Government of Malawi), for encouragement and permission to conduct the study.

Reprints: Susan Lewallen, MD, British Columbia Centre for Epidemiologic and International Ophthalmology, University of British Columbia, St Paul's Hospital, 1081 Burrard St, Vancouver, British Columbia, Canada V6Z 1Y6.

REFERENCES

1. World malaria situation 1990. *World Health Stat Q.* 1992;445:257-266.
2. *Malaria Research: An Audit of International Activity.* London, England: Wellcome Trust; 1996.
3. Lewallen S, Taylor TE, Molyneux ME, Will BA, Courtright P. Ocular fundus findings in Malawian children with cerebral malaria. *Ophthalmology.* 1993;100:857-861.
4. Lewallen S, Bakker H, Taylor TE, Wills BA, Courtright P, Molyneux ME. Retinal findings predictive of outcome in cerebral malaria. *Trans R Soc Trop Med Hyg.* 1996;90:144-146.
5. Sipperly JO, Quigley HA, Gass JDM. Traumatic retinopathy in primates: the explanation of commotio retinae. *Arch Ophthalmol.* 1978;96:2267-2273.
6. Hayes KC. Retinal degeneration in monkeys induced by deficiencies of vitamin E or A. *Invest Ophthalmol.* 1974;13:499-510.
7. Sommer A, West KP Jr. *Vitamin A Deficiency: Health, Survival, and Vision.* New York, NY: Oxford University Press; 1996.
8. Thurnham DI, Singkamani R. The acute phase response and vitamin A status in malaria. *Trans R Soc Trop Med Hyg.* 1991;85:194-199.
9. Natadisastra G, Wittpenn JR, Muhilal, West KP, Mele L, Sommer A. Impression cytology: a practical index of vitamin A status. *Am J Clin Nutr.* 1988;48:695-701.
10. Fuchs J, Ausayakhun S, Ruckphaopunt S, Tansuhaj A, Suskind RM. Relationship between vitamin A deficiency, malnutrition, and conjunctival impression cytology. *Am J Clin Nutr.* 1994;60:293-298.
11. *Assessment of Vitamin A Status and Impression Cytology: Training Manual.* Baltimore, Md: International Center Epidemiologic and Preventive Ophthalmology; 1988.
12. Arroyave G, Chichester CO, Flores H, et al. *Biochemical Methodology for the Assessment of Vitamin A Status: Report of the International Vitamin A Consultative Group (IVACG).* Washington, DC: Nutrition Foundation; 1982.
13. Hing TK. Further contributions to the fundus xerophthalmicus. *Ophthalmologica.* 1965;150:219-238.
14. Hero M, Harding S, Riva CE, Winstanley PA, Peshu N, Marsh K. Photographic and angiographic characteristics of retinal appearances in severe malaria in Kenyan children. *Arch Ophthalmol.* 1997;115:997-1003.
15. Lewallen S, Will BA. Retinal haemorrhage in children with malaria. *Lancet.* 1993;341:442.

Correction

Error in Table Data. In the article titled "MSL-109 Adjuvant Therapy for Cytomegalovirus Retinitis in Patients With Acquired Immunodeficiency Syndrome" published in the December 1997 issue of the ARCHIVES (*Arch Ophthalmol.* 1997;115:1528-1536), the reported values for the "Increase in retinal area involved by CMV" given in the "Other Visual Outcomes" subsection on page 1532 and in Table 3 on page 1534 are incorrect. The correct values for the MSL-109 group, placebo group, and *P* are 1.49, 1.55, and .93, respectively.

140

IRON DEFICIENCY AND ANEMIA

© International Epidemiological Association 1998 Printed in Great Britain International Journal of Epidemiology 1998;27:530–537

Epidemiological evidence for a differential effect of hookworm species, *Ancylostoma duodenale* or *Necator americanus*, on iron status of children

M Albonico,[a] RJ Stoltzfus,[b] L Savioli,[c] JM Tielsch,[d] HM Chwaya,[e] E Ercole[f] and G Cancrini[g]

Background The hookworms, *Ancylostoma duodenale* and *Necator americanus*, cause significant gastrointestinal blood loss. In clinical studies, greater blood losses have been reported with *A. duodenale*. However, there has been no evidence that endemic *A. duodenale* infection has greater impact than *N. americanus* infection on the iron status of populations.

Methods In a sample of 525 school children in Pemba Island, Tanzania, we compared the degree of anaemia and iron deficiency associated with the two hookworm species at the individual and community (i.e. school) levels. Multiple regression was used to control for infection intensities and other child characteristics.

Results In the 492 children with hookworm positive faecal cultures, haemoglobin and ferritin concentrations decreased with increasing proportions of *A. duodenale*. Among children with only *N. americanus* larvae, the prevalence of anaemia was 60.5% and the prevalence of ferritin <12 μg/l was 33.1%, while in children with ≥50% *A. duodenale* larvae, the respective prevalences were 80.6% and 58.9%. When children were grouped by the prevalence of *A. duodenale* at the school level, children from high prevalence (≥20%) schools had signficantly worse iron deficiency and anaemia than children from low prevalence schools.

Conclusions The species of hookworm being transmitted in a community influences the burden of iron deficiency anaemia in the community, and should be considered in prioritizing and planning programmes for hookworm and anaemia control.

Keywords Hookworms, *Ancylostoma duodenale*, *Necator americanus*, school children, iron deficiency, anaemia

Accepted 7 October 1997

Hookworms infect an estimated 1.3 billion people globally.[1] Iron-deficiency anaemia caused or exacerbated by intestinal blood loss is the most outstanding feature of hookworm infection.[2,3] Blood loss is caused when hookworms attach to and feed from the intestinal mucosa, and the magnitude of blood loss is directly related to the number of adult worms residing in the intestine.[4] The balance between iron intake and iron loss from intestinal bleeding determines the risk and severity of iron-deficiency anaemia from hookworm infection.[5]

There are primarily two species of hookworms that infect humans; *Ancylostoma duodenale* and *Necator americanus*.[6] *Necator americanus* tends to thrive in more tropical climates, while *A. duodenale* favours cooler, drier climates. However, their geographical distributions overlap considerably, and both species are endemic to many areas.[2] Hookworms are transmitted when hookworm eggs excreted in human faeces are incubated in appropriate soil conditions, hatch into larvae and enter a human host through the skin. In contrast to *N. americanus*, *A. duodenale* can infect humans by the oral as well as the percutaneous route[7] and may undergo arrested development in human organ systems in its larval stage.[8]

[a] Division of Communicable Diseases, World Health Organization, Geneva, Switzerland.

[b] Center for Human Nutrition, School of Hygiene and Public Health, The Johns Hopkins University, 615 N Wolfe St, Baltimore, MD 21205, USA.

[c] Division of Control of Tropical Diseases, World Health Organization, Geneva, Switzerland.

[d] Department of International Health, The Johns Hopkins School of Public Health, Baltimore, USA.

[e] Ministry of Health, Zanzibar, United Republic of Tanzania.

[f] Pemba Helminth Control Programme, Zanzibar, United Republic of Tanzania.

[g] Department of Parasitology, University 'La Sapienza', Rome, Italy.

Reprint requests to: Rebecca J Stoltzfus.

142

From a morbidity standpoint, the most important difference between the two species is that *A. duodenale* causes approximately five times greater blood loss than *N. americanus*.[2] This has been demonstrated in clinical studies in which isotopically labelled red blood cells were injected into hookworm-infected individuals, and the faecal excretion of the label was measured before and after deworming. Estimates from individual studies have found 2- to 10-fold higher blood losses with *A. duodenale* than with *N. americanus*.[4]

However, there has not been evidence that endemic *A. duodenale* infection has greater impact than *N. americanus* infection on the iron status of populations. The population biology of hookworms,[9] the host-parasite interaction,[10] and iron absorption in humans[11] may adapt in ways that prevent the burden of hookworm anaemia from becoming overly great in stable human populations.[12] Such compensatory strategies may prevent species related differences in blood loss per hookworm from manifesting into differences in the prevalence and intensity of iron-deficiency anaemia in human populations.

Because of the difficulty of measuring actual numbers of worms in population studies, most studies of hookworm infection and iron-deficiency anaemia in communities have used faecal egg counts to determine the presence and severity of hookworm infection. By this method, the two hookworm species cannot be distinguished, because their eggs have similar microscopic appearance.[2] Thus, there has been no epidemiological evidence that the species of hookworm being transmitted in a community has a meaningful impact on the iron status of the population.

We have studied the epidemiology of hookworm infection and iron-deficiency anaemia in schoolchildren in Pemba Island, Zanzibar.[13] Hookworms are highly endemic on the island,[14] and both species were believed to be present.[15] In the course of an ongoing evaluation of the impact of school-based deworming on children's nutritional status, we determined the species of hookworm infection in a sample of 525 children from 10 randomly selected communities. Infection intensities and iron status were also assessed. Our objectives were: (1) to describe the distribution of the two hookworm species across the island, (2) to determine whether children infected with *A. duodenale* have worse iron status than those infected with *N. americanus*, and (3) to demonstrate whether communities where *A. duodenale* is highly endemic have greater burdens of iron-deficiency anaemia than communities primarily infected with *N. americanus*, controlling for other factors.

Methods

Study area and sample

Pemba Island, the smaller of the two islands of Zanzibar, United Republic of Tanzania, lies 40 km off the East African coast, 5°S of the equator and is classed as a continental island with humid tropical climate. The northeast monsoon blows from October to December and is characterized by short rains. The southwest monsoon brings the long rains from mid-March to mid-May. The temperatures rarely exceed 34° or fall below 21°C.

Pemba is densely populated with 330 inhabitants per km^2. The island is divided into four districts of roughly equal size. About 15 000 people live in each of the three main towns of Wete, Chake-Chake and Mkoani, the rest of the population being scattered in numerous villages. The economy of the inhabitants is mainly based on farming and fishing. The primary crops consist of subsistence agriculture, with some clove and coconut plantations.

The sample for the present study was selected from children included in the baseline evaluation survey for the Zanzibar school-based deworming programme. For the evaluation, 12 schools were randomly selected stratified by district, and 3605 children in morning classes of grades 1–4 in those schools participated in the survey.[13] The sample for the present study included 570 children, drawn as a systematic random subsample. Specifically, every seventh child surveyed from 10 of the 12 schools was included. Children from the first school were not included because the method for culturing hookworm larvae was not yet in place, and children from the last school were not included because we had already exceeded our intended sample size of 500 children.

The study was approved by the internal review boards of The Johns Hopkins University, the Ministry of Health of Zanzibar, and the World Health Organization.

Assessments

Survey data were collected in the schools by specially trained staff of the Ministry of Health of Zanzibar. On the day before the survey, plastic containers were distributed to each class and the children were asked to bring a stool sample to school the next day. Over 95% of children returned a faecal sample. Faecal hookworm egg counts were determined by the Kato-Katz technique.[16] To determine hookworm species, cultures were successfully obtained for 525 of the 570 children selected for the study. The Harada-Mori technique[17] modified according to Garcia & Bruckner[18] was used in order to increase the efficiency of the culture method. Approximately 1 g faeces was mixed with active granular charcoal, and some water was added in the case of hard stools, to obtain an homogeneous paste. Two microscope slides were folded in a piece of absorbent paper and placed in a Petri dish with a diameter of 9 cm. The stool specimens were smeared carefully on the surface of the absorbent paper and 5–6 ml of distilled water were added to the Petri dish, without reaching the stool level. The cultures were covered and labelled and left at room temperature (26–30°C) in a shady place. The positivity of the cultures was checked with a dissecting microscope and after 7–10 days the cultures were transferred to a test tube. The larvae were killed by heating (100°C) in water for 2–3 min and then fixed with 10% formalin. The sediment was measured and a representative sample (usually 1/3 or 1/4) was examined for larvae that were identified and counted. The counts were then multiplied by 3 or 4 according to the proportion of sediment examined.

Urine samples were collected from 100% of children, and microhaematuria was diagnosed using Hemastix test strips (Ames Laboratories, Inc., Elkhart, IN). In this population microhaematuria screens for urinary schistosomiasis (*Schistosoma haematobium* infection) with 69% sensitivity and 89% specificity.[19]

Blood samples were collected by venipuncture from all children to assess iron status. Haemoglobin was determined by the HemoCue method (HemoCue AB, Angelhom, Sweden) and erythrocyte protoporphyrin was determined using a front-face haematofluorometer (Aviv Biomedical Inc., Lakewood, NJ). A thin blood film was stained with Giemsa and malaria parasites

143

were quantitated against leukocytes using standard methods.[20] The remaining blood was allowed to clot at room temperature, and serum was collected by centrifugation. Sera were stored at 10°C for up to 8 weeks, transported to Baltimore in liquid nitrogen and stored at −70°C for up to 4 months. Serum ferritin was determined on 442 samples (84% of study children) using a fluorescence-linked immunoassay (DELFIA System by Wallac, Inc., Gaithersburg, MD). Missing data for serum ferritin were mainly due to mislabelling of specimens, and losses in transport.

Statistical analysis

Distributions of faecal egg counts, serum ferritin and erythrocyte protoporphyrin were skewed to high values, so geometric means are presented. Anaemia was defined as haemoglobin <11 g/dl. This is lower than the WHO-recommended cutoff for anaemia, but a lower cutoff screens more efficiently for iron-deficiency anaemia in black populations.[21] A haemoglobin cutoff of 9 g/dl was used to define moderate-severe anaemia. Cutoffs for protoporphyrin are not well-defined. In these analyses values >120 μmol/mol heme were considered indicative of severe iron-deficient erythropoiesis. Serum ferritin <12 mg/l was used to indicate exhausted iron stores.

The prevalences of *A. duodenale* and *N. americanus* infections were examined for the entire sample, and by school. To compare the effect of the two species in individual children, children with hookworm positive larval cultures were grouped into those with only *N. americanus* larvae, those with 1–50% *A. duodenale* larvae, and those with >50% *A. duodenale* larvae. The latter group includes 11 children with apparently pure *A. duodenale* infections (i.e. no *N. americanus* larvae were found).

Necator americanus was highly prevalent in all communities surveyed, but the prevalence of *A. duodenale* ranged from 2% to 88% (Figure 1). To examine the effect of species on community burden of iron-deficiency anaemia, children in four schools with >20% prevalence of *A. duodenale* were compared to children in six schools with <20% prevalence.

In both individual and community level analyses, multiple regression was used to test the effect of hookworm species on measures of iron status. In individual level analyses the hookworm species variable was an ordinal variable with three values: 0 if pure *N. americanus*, 1 if 1–50% *A. duodenale*, and 2 if >50% *A. duodenale*. In the community level analyses, the hookworm species variable represented low prevalence versus high prevalence schools. The models included all child characteristics that differed between hookworm species groups, or that we knew to be associated with iron status from previous analyses.[13] Adjusted mean values and standard deviations for iron status measures were calculated by analysis of variance.

Whether and how to control for the intensity of hookworm infection presented a dilemma. Because the blood loss caused by hookworm infection is directly proportional to the number of adult worms in the intestine, differences in intensity (i.e. worm burdens) of *A. duodenale* and *N. americanus* infections could confound the comparison of iron status between individuals or communities infected with the two species. We measured intensity of infection by faecal egg counts, but these cannot be directly compared between the two species because *A. duodenale* produces 2–3 times more eggs per adult worm than does *N. americanus*. Furthermore, there was not a simple way to correct the faecal egg counts from the Kato-Katz method to reflect the relative worm burdens of the two species.[2]

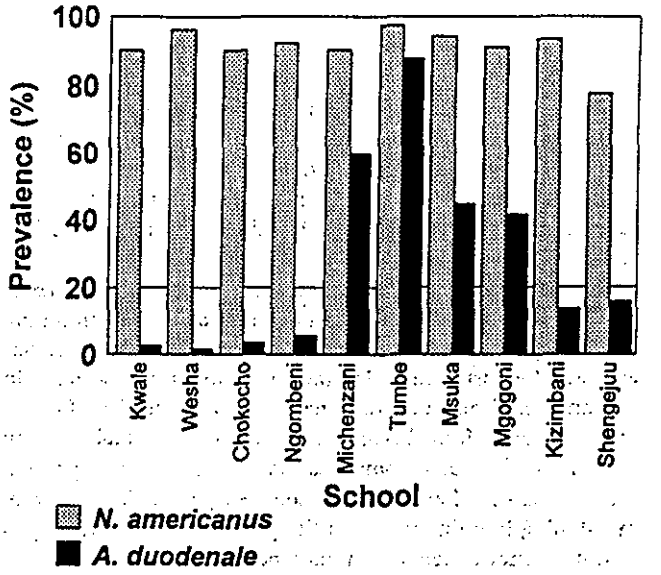


Figure 1 Prevalence of *N. americanus* and *A. duodenale* infections in children from 10 schools on Pemba Island, Tanzania. Number of children surveyed in each school ranges from 35 to 60.

Because a given number of eggs/g faeces represents fewer *A. duodenale* worms than *N. americanus* worms, controlling for hookworm eggs/g faeces might bias against finding a true species-related difference in iron status. However, it protects us from attributing to species a difference in iron status that was truly caused by greater worm burdens. We carried out analyses with and without adjustment for hookworm eggs/g faeces, and found it changed the results very little. We present results of analyses with adjustment for hookworm eggs/g faeces, a conservative approach. To adjust for hookworm eggs/g faeces, an ordinal categorical variable was included in the multivariate regression models, coded as 0 if 0 eggs/g, 1 if 1–1999 eggs/g, 2 if 2000–3999 eggs/g, and 3 if 4000–5999 eggs/g, and 4 if ≥6000 eggs/g faeces. The multiple R^2 for the models were highest when we used the variable in this form, rather than geometric mean eggs/g or other categorical forms. Data were analysed using Systat statistical software (SPSS, Inc., Chicago, IL).

Results

Prevalence and geographical distribution of hookworm species

Based on the faecal cultures, 33 children (6.3%) were not infected with hookworms, 11 (2.1%) were infected with only *A. duodenale*, 340 (64.8%) were infected with only *N. americanus*, and 141 (26.9%) were infected with both species of hookworm (Table 1). The prevalence of hookworm infection based on faecal cultures was 93.7%, and based on Kato-Katz method was 96.2%. The overall agreement for hookworm positivity between the faecal culture and Kato-Katz methods was 91.4% (480/525). Only four of the 525 children studied, less than 1% of the sample, were hookworm negative by both methods.

144

Table 1 Number of faecal samples diagnosed with hookworm infection by Kato-Katz method and faecal culture method

	Faecal culture result				
		Positive for			
Kato-Katz result	Negative	*A. duodenale* only	*N. americanus* only	*A. duodenale* + *N. americanus*	All
Negative	4	3	11	2	20 (3.8%)[a]
Positive	29	8	329	139	505 (96.2%)
All	33 (6.3%)[b]	11 (2.1%)	340 (64.8%)	141 (26.9%)	525

[a] Column per cents.

[b] Row per cents.

The prevalence of *N. americanus* infection was uniformly high in all 10 schools surveyed, but the prevalence of *A. duodenale* varied greatly, from 2% to 88% (Figure 1). Four schools (Michenzani, Tumbe, Msuka, and Mgogoni) were considered highly endemic for *A. duodenale* with prevalences of 42–88%, while the remaining six schools had prevalences below 18%. The four schools with high prevalence of *A. duodenale* were located at opposite ends of the island, and did not share any obvious geographical characteristics (Figure 2). Because *A. duodenale* transmission may exhibit seasonality, the dates of the survey in each school are included in Figure 2. The heavy rains began as the survey team moved from Chokocho to Ngombeni school, and continued for the duration of the study. The four schools with high prevalence of *A. duodenale* were surveyed during the heavy rains, but so were Ngombeni, Kizimbani and Shengejuu which had low prevalences.

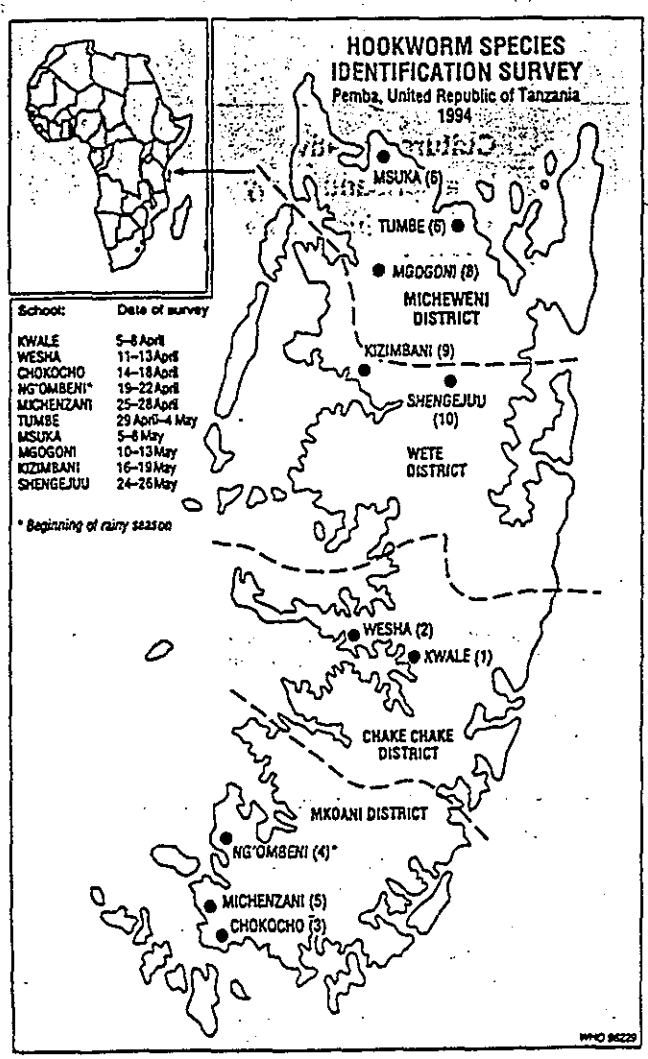


Figure 2 Map of Pemba Island, Tanzania, showing geographic location of the 10 survey schools and the dates of the survey in each school

Hookworm species and individuals' iron status

The characteristics of children with only *N. americanus* infection did not differ significantly from those infected with *A. duodenale* (Table 2). The children were on average 10 years old, and about half were girls. About one-fourth of children had microhaematuria, an indicator of urinary schistosomiasis, and around 60% were positive for *Plasmodium falciparum*. *Trichuris trichiura* and *Ascaris lumbricoides* infections were common. The prevalence and intensity of these infections did not differ significantly between hookworm species groups.

The prevalence and intensity of hookworm infection, assessed by the Kato-Katz method, did not differ significantly between the hookworm species groups, but were significantly lower in children with negative faecal cultures (Table 2). The distribution of faecal egg counts assessed by the Kato-Katz method was similar between the hookworm species groups (Figure 3).

By all indicators, the iron status of children infected with *A. duodenale* was worse than those infected with only *N. americanus*, and haemoglobin and serum ferritin values became progressively worse according to the percentage of *A. duodenale* larvae found (Table 3). Overall there is a consistent pattern of worse iron status in children with more *A. duodenale* infection. The differences between groups was most significant for serum ferritin, the indicator most specific to iron deficiency.

Hookworm species and community burden of iron deficiency and anaemia

Compared to children in schools with low prevalence of *A. duodenale*, children in the four schools with high *A. duodenale* prevalence were slightly older and had a higher prevalence of microhaematuria (Table 4). The prevalence and intensity of *T. trichiura* or *A. lumbricoides* infection did not differ between children in the two groups of schools. However, the prevalence of hookworm infection and hookworm faecal egg counts were significantly higher in children in the schools with high *A. duodenale* prevalence. The difference in the hookworm faecal egg counts was apparent both in the mean values (Table 4) and in the distribution of values (Figure 4).

After adjusting for these differing characteristics between the groups, children in the high prevalence schools had significantly

Table 2 Characteristics of study children by species of hookworm infection determined by faecal cultures

Characteristic	Negative culture (n = 33)	*N. americanus* larvae only (n = 340)	*A. duodenale*[a] <50% of larvae (n = 113)	*A. duodenale*[a] ≥50% of larvae (n = 39)
Age (years)	10.4 ± 1.5[b]	10.5 ± 1.9	11.0 ± 2.1	10.5 ± 2.6
Sex (% girls)	42.4%	51.0%	45.9%	48.7%
Microhaematuria[c]	24.2%	23.4%	24.3%	23.1%
Malaria parasitaemia	60.6%	63.3%	60.7%	54.1%
T. trichuria				
prevalence	93.9%	97.0%	96.4%	100.0%
eggs/g faeces	525 (73, 3763)[d]	633 (117, 3439)	577 (108, 1856)	602 (193, 1882)
A. lumbricoides				
prevalence	72.7%	71.5%	71.2%	76.9%
eggs/g faeces	239 (6, 9072)	240 (6, 9691)	233 (6, 9576)	326 (10, 10 290)
Hookworms[e]				
prevalence	87.9%	96.8%	98.2%	92.3%
eggs/g faeces	258 (44, 1499)	608 (102, 3607)	844 (188, 3790)	420 (56, 3168)

[a] Includes 11 samples in which only *A. duodenale* larvae were found, and 141 samples in which both species were found.
[b] Mean ± SD.
[c] An indication of urinary schistosomiasis.
[d] Geometric mean (−1 SD, +1 SD).
[e] Prevalence and intensity of hookworm infection by Kato-Katz method.

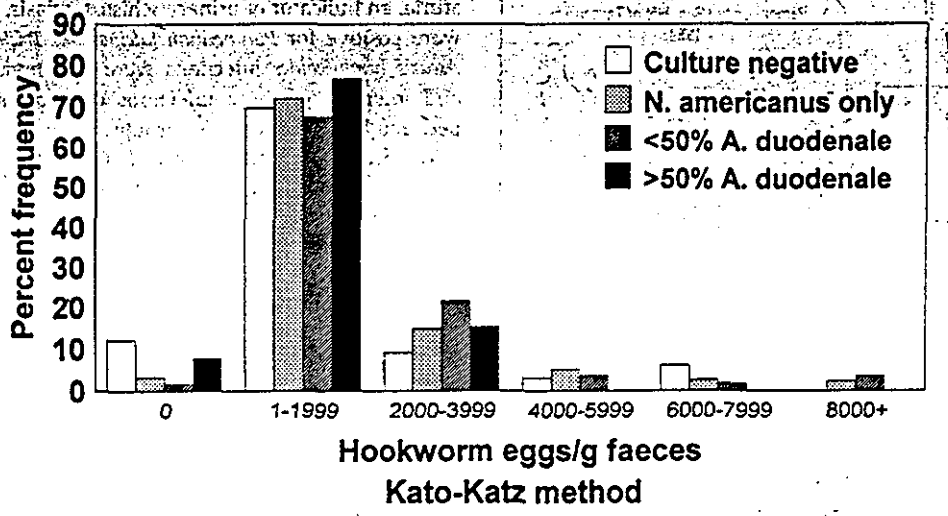


Figure 3 Distributions of hookworm faecal egg counts by hookworm species category determined by larval culture. Numbers of children in species categories: culture negative, 33; *N. americanus* only, 340; <50% *A. duodenale*, 113; ≥50% *A. duodenale*, 39. Distributions are not significantly different by χ^2 test

worse iron status by a variety of measures (Table 5). Haemoglobin and ferritin concentrations were lower, and protoporphyrin concentrations were higher in children from schools where *A. duodenale* was highly endemic.

Discussion

These results indicate that infection with *A. duodenale* in children and heavy transmission of *A. duodenale* in school communities is associated with a greater burden of iron-deficiency anaemia than infection with *N. americanus*. Previous claims of the greater pathogenicity of *A. duodenale* have been based on highly varying estimates of greater blood loss in *A. duodenale* infection. To our knowledge, this is unique epidemiological evidence that the previously measured difference in blood loss translates into a greater burden of hookworm-related anaemia in individuals and communities infected with *A. duodenale*.

The degree of iron-deficiency anaemia caused by hookworm infection depends on the number of adult worms inhabiting the gut, the duration of infection, the iron stores and intake of the infected individual[12,22–24] and, as we have attempted to demonstrate, the species of hookworm. Ideally, proof of species

Table 3 Iron status of hookworm-infected children according to degree of *A. duodenale* infection determined by faecal culture

Iron status indicator[a]	*N. americanus* larvae only (n = 340)	*A. duodenale* <50% of larvae (n = 113)	*A. duodenale* ≥50% of larvae (n = 39)
Haemoglobin (g/dl)			
Mean ± SD[b]	10.4 ± 1.5	10.3 ± 1.5	9.8 ± 1.5
% <11[b]	60.5	64.5	80.6
% <9	14.0	18.3	22.8
Protoporphyrin (μmol/mol heme)			
Geometric mean (+1 SD, −1 SD)	97 (58, 162)	109 (65, 183)	103 (62, 173)
% >120	29.6	36.9	32.9
Ferritin (μg/l)			
Geometric mean (+1 SD, −1 SD)[c]	14.5 (7.7, 27.5)	13.4 (7.2, 25.4)	9.6 (5.1, 18.1)
% <12[c]	33.1	50.7	58.9

[a] Values are predicted from analysis of variance, adjusted for hookworm eggs/g faeces determined by Kato-Katz method, age, height-for-age Z score, malaria parasitaemia, and sex.

[b] Linear trend in indicator by hookworm species category significant, $P \leq 0.05$.

[c] Linear trend in indicator by hookworm species category significant, $P \leq 0.001$.

Table 4 Characteristics of study children in schools with low or high prevalence of *A. duodenale* infection determined by faecal cultures

Characteristic	*A. duodenale* prevalence: Low, <20% (6 schools, 308 children)	High, >20% (4 schools, 217 children)
Age (years)	10.4 ± 1.7[a,b]	11.0 ± 2.3
Sex (% girls)	48.5%	50.2%
Microhaematuria[c]	20.9%[d]	27.6%
Malaria parasitaemia	61.6%	62.3%
T. trichiura		
prevalence	96.7%	97.2%
eggs/g faeces	637 (110, 3689)[e]	575 (123, 2692)
A. lumbricoides		
prevalence	71.5%	72.6%
eggs/g faeces	234 (6, 9499)	259 (7, 9789)
Hookworms[f]		
prevalence	94.8%[b]	98.2%
eggs/g faeces	486 (69, 3439)[b]	811 (180, 3652)

[a] Mean ± SD.

[b] Significantly different from high prevalence schools, $P < 0.05$.

[c] An indication of urinary schistosomiasis.

[d] Significantly different from high prevalence schools, $P < 0.10$.

[e] Geometric mean (−1 SD, +1 SD).

[f] Prevalence and intensity of hookworm infection by Kato-Katz method.

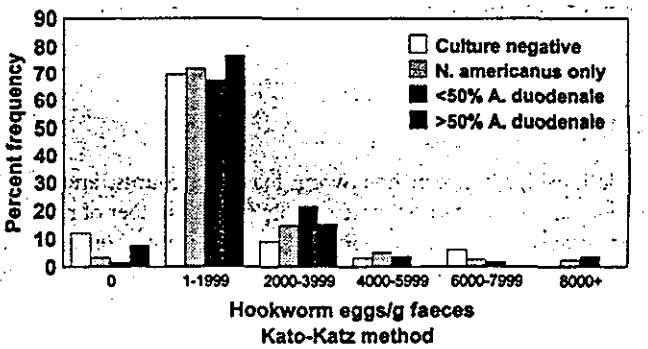


Figure 4 Distributions of hookworm faecal egg counts in children from schools with low (<20%) or high (≥20%) prevalence of *A. duodenale*. Numbers of children in low and high prevalence groups are given in Table 4. Distributions are significantly different by χ^2 test, $P = 0.041$

differences in the impact on iron status of individuals would be based on measurement of all of these factors. In reality, such a study is nearly impossible to conduct. To account for the duration of infection, one would have to begin with an uninfected individual, and follow longitudinally the course of infection both in terms of species and number of worms, the iron intake and bioavailability of the diet, and the iron status of the individual. Such a study would illuminate our understanding of iron balance in hookworm infection, the role of hookworm species, and particularly the adaptations that might occur as haemoglobin levels become acutely low. However, the technical and ethical difficulties of such an observational study are likely prohibitive.

In weighing the strength of the present epidemiological evidence, it is useful to consider each of the four factors listed above. We measured intensity of infection by faecal egg counts, not by expulsion and enumeration of adult worms. The relationship between faecal counts and number of worms is different for the two species by a factor that is not precisely known. Adult female *A. duodenale* produce more eggs than do *N. americanus*, and the male to female ratio of adult worms is higher for *A. duodenale* than for *N. americanus*.[9] This is problematic since we would like to compare the effect of species at similar levels of worm burdens. One could try to account for the differences by mathematically modelling the relationships, but 'the arithmetic

Table 5 Iron status of Zanzibari children by prevalence of *A. duodenale* in the school

Iron status indicator[a]	*A. duodenale* prevalence: Low, <20% (6 schools, 308 children)	High, >20% (4 schools, 217 children)
Haemoglobin (g/dl)		
Mean ± SD	10.5 ± 1.5[b]	10.2 ± 1.5
% <11	58.9	65.1
% <9	12.7[c]	18.3
Protoporphyrin (μmol/mol heme)		
Geometric mean (+1 SD, −1 SD)	93 (55, 156)[d]	109 (65, 184)
% >120	27.1[b]	35.2
Ferritin (μg/l)		
Geometric mean (+1 SD, −1 SD)	14.8 (7.8, 28.0)[c]	13.1 (6.9, 24.9)
% <12	31.0[d]	48.4

[a] Values are predicted from analysis of variance, adjusted for hookworm eggs/g faeces determined by Kato-Katz method, age, height-for-age Z score, malaria parasitaemia, haematuria and sex.

[b] Significantly different from high prevalence schools, $P < 0.05$.

[c] Significantly different from high prevalence schools, $P < 0.10$.

[d] Significantly different from high prevalence schools, $P < 0.001$.

relationships between hookworm load, egg counts, and blood losses are difficult to estimate reliably[2] for a number of reasons. By simply including categories of hookworm eggs/g faeces in our analyses of the species variables, we have compared the iron status of individuals and communities within those categories and thus have likely underestimated the impact of greater blood loss per worm with *A. duodenale*. Although an unbiased estimate of the species difference based on numbers of worms is desirable, any mathematical model would be highly speculative, and this answer awaits a future study in which worms are expelled and counted. In the meantime, it is reasonable to infer that the true effect on iron status by the different species is at least as great as our results would indicate.

We did not measure duration of infection nor iron intake and bioavailability. This does not threaten our inferences unless duration of infection or iron intake were different for children or school communities in different species categories. We can think of no scenario in which the duration of infection might have differed by species, since both species likely have been endemic on Pemba Island for many years and children's risk of exposure with age is not known to differ for the two species.

It is somewhat more probable that dietary iron intake might be associated with species in our community level analyses. If children infected with *A. duodenale* or schoolchildren in communities with high levels of *A. duodenale* infection consume less iron than their counterparts infected with *N. americanus*, then our results could be spurious. Lacking dietary intake data, we cannot prove that this is not the case. However, we consider it to be unlikely that in this environment where essentially all children are infected with hookworms, the determinants of exposure to *A. duodenale* as opposed to *N. americanus* would also be associated with poor diet.

The two approaches we took to address the question of species impact have complementary strengths and weaknesses. The comparison of individuals by degree of *A. duodenale* infection does not associate the species variable with school, an advantage because school communites may vary in level of poverty and thus diet. Rather children are compared to other children, some of whom come from the same school community. Our results from this analysis are also strengthened by the dose-reponse pattern seen in haemoglobin and ferritin values by degree of *A. duodenale* infection (Table 3).

The comparison of children grouped by community prevalence of *A. duodenale* infection represents the practical question that confronts public health planners. Are communities or areas where *A. duodenale* is highly endemic in greater need of intervention than communities where *N. americanus* predominates? Based on our results, the answer to this question is 'Yes.' The strength of this analysis is that in reality the risk of *A. duodenale* infection (or the force of transmission of that parasite) is more characteristic of a community than of an individual. The children in school communities with high prevalence of *A. duodenale* who happened not to be infected with that species at the time of our survey, have likely experienced *A. duodenale* in the past or will do so in the future. The community also represents the level at which public health decisions are made. The predominate hookworm species being transmitted may be an appropriate means of characterizing and targeting communities, but will rarely influence the course of treatment of an individual.

Most importantly, the weight of our evidence is strengthened by the consistency of the findings from complementary approaches to the problem. We conclude that, as predicted from earlier clinical studies, the species of hookworm being transmitted in a community influences the burden of iron-deficiency anaemia in the community.

The full implications of hookworm species for the planning and implementation of interventions are yet to be determined. Our results suggest that where iron-deficiency anaemia is a public health problem, hookworm control is particularly urgent if *A. duodenale* is the predominant species. However, this should not be interpreted to mean that endemic *N. americanus* infection is of low priority. We and others have demonstrated previously that hookworms can be an important cause of anaemia in populations where *N. americanus* predominates.[23,24] Where anaemia is prevalent and hookworms are known to be endemic, lack of knowledge about hookworm species should not impede interventions.

A potentially important question is whether the optimal regimen for anthelminthic control of hookworms in populations differs for the two species. Because transmission of *A. duodenale* has a distinctly seasonal pattern in some environments,[8] it is possible that the efficacy of public health deworming regimens would depend on the seasonal timing of treatments. It is also possible that the rate of reinfection following treatment differs for the two species, with implications for the frequency of treatments. Further epidemiological research is needed to answer these questions.

Acknowledgements

The authors thank the Helminth Control Team, especially Mr Faki BF, for their excellent work in collecting these data.

148

The work was supported by cooperative agreement DAN-5116-1-00-8051-00 between The Johns Hopkins University and the Office of Health and Nutrition, United States Agency for International Development.

References

[1] Warren KS, Bundy DAP, Anderson RM *et al.* Helminth infection. In: Jamison DT, Mosley WH, Measham AR, Bobadilla JL (eds). *Disease Control Priorities in Developing Countries.* New York: Oxford University Press, 1993, pp.131–60.

[2] Pawlowski ZS, Schad GA, Stott GJ. *Hookworm Infection and Anaemia: Approaches to Prevention and Control.* Geneva: World Health Organization, 1991.

[3] Miller TA. Hookworm infection in man. *Adv Parasitol* 1978;**17**:315–84.

[4] Roche M, Layrisse M. The nature and causes of 'hookworm anemia'. *Am J Trop Med Hyg* 1966;**15**:1031–100.

[5] Gilles HM, Watson Williams EJ, Ball PAJ. Hookworm infection and anaemia: an epidemiological, clinical, and laboratory study. *Q J Med* 1964;**33**:1–24.

[6] Stephenson LS. *Impact of Helminth Infections on Human Nutrition.* New York: Taylor & Francis, 1987.

[7] Schad GA. Presidential address. Hooked on hookworm: 25 years of attachment. *J Parasitol* 1991;**77**:177–86.

[8] Schad GA, Chowdhury AB, Dean CG *et al.* Arrested development in human hookworm infections: an adaptation to a seasonally unfavorable external environment. *Science* 1973;**180**:502–04.

[9] Anderson RM, Schad GA. Hookworm burdens and faecal egg counts: an analysis of the biological basis of variation. *Trans R Soc Trop Med Hyg* 1985;**79**:812–25.

[10] Pritchard DI. The survival strategies of hookworms. *Parasitology Today* 1995;**11**:255–59.

[11] Cook JD. Adaption in iron metabolism. *Am J Clin Nutr* 1990;**51**: 301–08.

[12] Crompton DWT, Whitehead RR. Hookworm infections and human iron metabolism. *Parasitology* 1993;**107**:S137–145.

[13] Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KJ, Albonico M, Savioli L. Epidemiology of iron deficiency anemia in Zanzibari school children. *Am J Clin Nutr* 1997;**65**:153–59.

[14] Renganathan E, Ercole E, Albonico M *et al.* Evolution of operational research studies and development of a national control strategy against intestinal helminths in Pemba Island experience, 1988–1992. *Bull World Health Organ* 1995;**73**:183–90.

[15] de Carneri I, Albonico M, Di Matteo L, Savioli L, Kisumku UM. Sia Necator sia Ancylostoma parassitano l'uomo a Pemba (Zanzibar). *Parassitologia* 1992;**34(Suppl.1)**:190.

[16] World Health Organization. *Bench Aids for the Diagnosis of Intestinal Parasites.* Geneva: WHO, 1994.

[17] Harada Y, Mori O. A new method for culturing hookworm. *Yonago Acta Medica* 1955;**1**:177–95.

[18] Garcia LS, Bruckner DA. *Diagnostic Medical Parasitology.* New York: Elsevier, 1988.

[19] Savioli L, Hatz C, Dixon H, Kisumku UM, Mott KE. Control of morbidity due to *Schistosoma haematobium* on Pemba Island: egg excretion and haematuria as indicators of infection. *Am J Trop Med Hyg* 1990;**43**:289–95.

[20] Trape JF. Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. *Trans R Soc Trop Med Hyg* 1985;**79**:181–84.

[21] Johnson-Spear MA, Yip R. Hemoglobin difference between black and white women with comparable iron status: justification for race-specific anemia criteria. *Am J Clin Nutr* 1994;**60**:117–21.

[22] Gilles HM. Selective primary health care: Strategies for control of disease in the developing world. XVII. Hookworm infection and anemia. *Rev Infect Dis* 1985;**7**:111–18.

[23] Stoltzfus RJ, Albonico M, Chwaya HM *et al.* HemoQuant determination of hookworm-related blood loss and its role in iron deficiency in African children. *Am J Trop Med Hyg* 1996;**54**:399–404.

[24] Layrisse M, Roche M. The relationship between anemia and hookworm infection. *Am J Hyg* 1964;**79**:279–301.

almost equal accuracy at any SF (6). How well did R alone at 50 kHz predict ECW? Reference measures of both TBW and ICW had been measured.

The prediction of TBW with use of R is dependent on a high intercorrelation between ECW and TBW (6) because only a portion of ICW is measured (2). Furthermore, the proportion of ICW measured at 50 kHz varies with characteristic frequency, which changes when the ECW, ICW, or cell membranes change (2, 7). The ECW and ICW resistivities differ by a factor of 3–4 (2). To predict TBW with an SF resistance requires that TBW resistivity be known and remain constant. How can it remain constant when a simple change in the ratio of ECW to ICW will change it (2, 7)?

Kotler et al made the statement that a "logarithmic transformation" was more representative of the body's complex shape (1). Kotler's group discovered randomly what we have been reporting since 1992, that the relation between impedance and body water is nonlinear because of mixture effects (that is, conductor-nonconductor interactions) (2). The exponent Kotler et al discovered statistically for height (Ht) was close to the 1.5 exponent we reported in 1992 by using Hanai mixture theory (2). We found that $Ht^{1.5}$ provided a simple form of mixture theory but relied on differences in body composition being purely due to differences in fluid rather than differences in both conductor and nonconductor. By developing a new equation that incorporated total object volume, we found that both conductor and nonconductor effects were accounted for (2).

Kotler et al used TBK and BCM interchangeably throughout their paper, reported no absolute values for TBK or BCM predicted by X_P, and did not report exactly how BCM was determined (1). The relation between TBK and BCM is derived only from the ICW-BCM relation. Without a report of how BCM was determined, this study cannot be replicated. Kotler et al assumed that if the regression coefficients, SEEs, means, and SDs for the model and validation groups were not different statistically, the derived model was valid. Final results were reported by using equations derived from the entire group. Although correlation and SEE values for the model and validation groups were reported, the regression coefficients and mean offsets were not. Thus, the cross-validation reported could not be fully evaluated. If the parameters of an equation vary randomly and are sample dependent, the scientific basis of the equation can be challenged and it would be poor at detecting change. It is curious that the scaling coefficients (statistically derived ICW resistivity) reported for the entire group were 59.06 for males and 1.30 for females (1). Such a difference between males and females is not realistic. The predictions across the board were significantly improved when the sexes were combined. That there was significant random error is supported by Kotler et al's Figure 1 (1), in which there were many outliers, some out by as much as 50%. Possibly this explains why only correlations and SEE percentages were reported.

Impedance is frequency dependent, so how well did X_P at different frequencies predict BCM? Was the best prediction provided by 50 kHz? If the Cole model (3) and basic alternating circuit theory have any validity, X at any SF, either parallel or series, will be affected by all of the elements in the tissue (that is, R_E, R_I, C_m, and α). What is the effect of a change in each of these variables on X_P? If X_P does not physically relate to TBK or is reliant on the elements in the tissue having relative uniformity between individuals, its ability to measure change will be poor. Because of high intercorrelation between variables, only data that conform to a plausible physical model (eg, Cole's model) should be considered (2). An investigation to answer many of these questions was conducted recently (7).

In closing, why is there such a need to make an SF measurement work? Whatever the reason, it does not appear to be based on science. Granted, when impedance was first proposed, neither microprocessors nor multifrequency measurements were available, but this is no longer the case. Human tissue is an electronic network consisting of multiple elements. Only in the body-composition field is an SF analysis of a multiple-component network considered. Although a multifrequency-modeling approach introduces new questions, an SF method is fraught with inherent error.

James R Matthie
Paul O Withers

Xitron Technologies, Inc
6295-D Ferris Square
San Diego, CA 92121
E-mail: jmatthie@xitron-tech.com

REFERENCES

1. Kotler DP, Burastero S, Wang J, Pierson RN Jr. Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: effects of race, sex, and disease. Am J Clin Nutr 1996;64(suppl):489S–7S.
2. De Lorenzo A, Andreoli A, Matthie J, Withers P. Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. J Appl Physiol 1997;82:1542–58.
3. Cole K. Membranes, ions and impulses: a chapter of classical biophysics. Berkeley, CA: University of California Press, 1972.
4. Lukaski HC. Biological indexes considered in the derivation of the bioelectrical impedance analysis. Am J Clin Nutr 1996;64(suppl): 397S–404S.
5. Segal KR, Burastero S, Chun A, Coronel P, Pierson RN Jr, Wang J. Estimation of extracellular and total body water by multiple-frequency bioelectrical-impedance measurement. Am J Clin Nutr 1991;54:26–9.
6. van Marken Lichtenbelt WD, Westerterp KR, Wouters L, Luijendijk SCM. Validation of bioelectrical-impedance measurements as a method to estimate body-water compartments. Am J Clin Nutr 1994;60:159–66.
7. Matthie J, Zarowitz B, De Lorenzo A, et al. Analytic assessment of the various bioimpedance methods used to estimate body water. J Appl Physiol 1998;84:1801–16.

Antenatal iron supplementation as a child survival strategy

Dear Sir:

A recent double-masked, placebo-controlled clinical trial examined the effect of daily supplementation with 100 mg elemental Fe during the third trimester of pregnancy on iron status of women and newborns in Niger (1). This study found that supplementation *1*) decreased the prevalence of maternal iron deficiency anemia at delivery and 3 mo postpartum, *2*) improved the iron status of infants at 3 mo, and *3*) increased birth length and

150

Apgar scores of infants. The authors also reported 8 fetal or neonatal deaths in the study, 7 in the placebo group and 1 in the iron-supplemented group, but surprisingly neglected to analyze these data or comment on their importance. The corresponding fetal or neonatal death rates were 7.1% and 1% in the 2 groups, respectively. I used Fisher's exact test (given the small sample size: 99 women in the iron-supplemented group and 98 in the placebo group) and found a significant difference ($P = 0.0349$) in the fetal and early neonatal death rates for women who received iron supplements compared with those who received placebo. The significance and relevance of these findings is in my opinion considerable given that there are few randomized, placebo-controlled studies examining the effect of iron supplementation on outcomes such as fetal and infant survival.

Most of the evidence regarding the effects of anemia, especially severe anemia, during pregnancy on birth outcomes is based on observational studies. Less is known about the pregnancy consequences of mild-to-moderate anemia, which constitutes most of the disease burden in developing countries (2). Iron supplementation during pregnancy has had varied success in different parts of the world, with little progress having been made against combating iron deficiency anemia (3). Most developing countries, however, do have national policies regarding supplementing women antenatally with iron (and folate). The study by Preziosi et al (1) provides evidence that iron supplementation during the third trimester of pregnancy in women whose mean hemoglobin concentrations were ≈100 g/L can reduce fetal and early infant mortality. These findings need to be emphasized to promote antenatal iron supplementation as a child survival strategy.

Parul Christian

Johns Hopkins University
School of Hygiene and Public Health
Division of Human Nutrition
615 North Wolfe Street
Baltimore, MD 21205
E-mail: pchristi@jhsph.edu

REFERENCES

1. Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr 1997; 66:1178–82.
2. Stoltzfus RJ. Rethinking anaemia surveillance. Lancet 1997;349:1764–6.
3. United Nations Administrative Committee on Coordination, Subcommittee on Nutrition (ACC/SCN). Second report on the world situation: global and regional results. Vol 1. Geneva: ACC/SCN, 1992.

Reply to P Christian

Dear Sir:

The issue raised by Christian concerning our paper (1) is well taken. We thank him for his remark concerning the effect of iron supplementation on outcomes such as fetal and early infant mortality. We agree that this point is particularly relevant from a public health viewpoint, and few data are available on the functional benefit of oral iron supplementation during pregnancy on the status of infants. Most published data based on observational studies, even when the studies were performed in large samples of pregnant women, generally suggest a relation between maternal iron deficiency anemia and fetal and infant survival (2). But the design of observational studies does not enable a conclusion as to the causal relation.

In our study, the objective was to assess, in pregnant women living in a tropical environment, the effect of iron supplementation during the last trimester of pregnancy on the iron status of mothers and newborns and on clinical and anthropometric indexes. The design of our randomized study was adapted to answer these questions, but not to study the effect of iron supplementation on fetal and neonatal deaths. It is true that we reported that fetal and neonatal deaths were less frequent in the iron-supplemented group than in the placebo group. It is also true that there was a statistically significant difference in the death rates between the 2 groups. But the small sample size of pregnant women included and the small number of fetal and neonatal deaths (a total of 8 deaths) suggest caution in interpretation of this result. We believe that additional studies, particularly large intervention trials with sufficient statistical power, are necessary to evaluate the relative role of maternal iron deficiency in fetal and newborn mortality.

Serge Hercberg
Paul Preziosi
Pilar Galan
Alain Prual

Institut Scientifique et Technique de la Nutrition et de l'Alimentation
CNAM Paris
2 rue Conté
75003 Paris
France

REFERENCES

1. Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr 1997; 66:1178–82.
2. Scholl T, Hediger ML, Fisher RL, Shearer JW. Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. Am J Clin Nutr 1992;55:985–8.

151

Effects of the Zanzibar school-based deworming program on iron status of children[1-3]

Rebecca J Stoltzfus, Marco Albonico, Hababuu M Chwaya, James M Tielsch, Kerry J Schulze, and Lorenzo Savioli

ABSTRACT We evaluated the effects of the Zanzibar school-based deworming program on the iron status of primary school children. Parasitologic and nutritional assessments were carried out at baseline, 6 mo, and 12 mo in 4 nonprogram schools (n = 1002), 4 schools in which students received twice-yearly deworming (n = 952), and 4 schools in which students received thrice-yearly deworming (n = 970) with 500 mg generic mebendazole. Schools were randomly selected for evaluation and allocated to program groups. Relative to no treatment, thrice-yearly deworming caused significant decreases in protoporphyrin concentrations and both deworming regimens caused marginally significant increases in serum ferritin concentrations. The average annual changes in protoporphyrin concentrations were −5.9 and −23.5 μmol/mol heme in the control and thrice-yearly deworming groups, respectively (P < 0.001). The average changes in ferritin concentration were 2.8 and 4.5 μg/L, respectively (P = 0.07). Deworming had no effect on annual hemoglobin change or prevalence of anemia. However, the relative risk of severe anemia (hemoglobin <70 g/L) was 0.77 (95% confidence limits: 0.39, 1.51) in the twice-yearly deworming group and 0.45 (0.19, 1.08) in the thrice-yearly deworming group. The effects on prevalence of high protoporphyrin values and incidence of moderate-to-severe anemia (hemoglobin <90 g/L) were significantly greater in children with >2000 hookworm eggs/g feces at baseline. We estimate that this deworming program prevented 1260 cases of moderate-to-severe anemia and 276 cases of severe anemia in a population of 30 000 schoolchildren in 1 y. Where hookworm is heavily endemic, deworming programs can improve iron status and prevent moderate and severe anemia, but deworming may be needed at least twice yearly. *Am J Clin Nutr* 1998;68:179–86.

KEY WORDS Humans, hookworms, schoolchildren, anemia, iron deficiency, deworming, Africa, helminth, anthelmintic drug, Zanzibar

INTRODUCTION

Iron deficiency anemia afflicts approximately half of the children in Asia and sub-Saharan Africa (1). However, outside of Europe and the Americas, relatively few interventions have been implemented to prevent childhood iron deficiency. The major strategies used to combat iron deficiency are supplementation and food fortification. The logistical difficulties of providing iron supplements to young children and the lack of centrally processed fortifiable foods outside of urban areas have prevented these interventions from reaching children in parts of Africa and Asia where anemia is most prevalent and severe.

Hookworms (*Ancylostoma duodenale* and *Necator americanus*) infect ≈880 million people globally and are most prevalent in Asia and sub-Saharan Africa (2). Hookworms cause chronic intestinal blood loss by attaching to the mucosa of the upper small intestine and ingesting tissue and blood. Blood loss occurs both from ingestion by the worm and through bleeding from the damaged mucosa (3). The quantity of blood lost is directly related to the intensity of the infection (4, 5). Where hookworm infections are prevalent and iron status poor, hookworm infection is an important cause of iron deficiency anemia, especially more severe anemia (6).

Apart from treating individuals with clinical hookworm-related anemia, hookworm control has not often been included in public health strategies to control iron deficiency anemia (7). This is in part because evaluations of the effects of hookworm control programs on iron deficiency in populations have been rare. An exception is an evaluation of anthelmintic therapy in combination with an iron-fortification pilot project in India, in which anthelmintic therapy significantly enhanced the benefit of iron-fortified salt at the population level (8).

School-based deworming is advocated as a highly cost-effective public health intervention (9) and might improve children's iron status in some populations. This benefit is most likely to be attained in populations where hookworm infections are prevalent and iron intakes poor. Blood loss may also occur in *Trichuris*

[1]From the Center for Human Nutrition and the World Health Organization Collaborating Center for Research on Intestinal Parasites and Human Nutrition, Department of International Health, The Johns Hopkins School of Public Health, Baltimore; the Schistosomiasis and Intestinal Parasites Unit, Division of Control of Tropical Diseases, World Health Organization, Geneva; and the Ministry of Health, Zanzibar, United Republic of Tanzania.

[2]Supported by cooperative agreement DAN-5116-1-00-8051-00 between The Johns Hopkins University and the Office of Health and Nutrition, United States Agency for International Development.

[3]Address reprint requests to RJ Stoltzfus, Center for Human Nutrition, Department of International Health, The Johns Hopkins School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205. E-mail: rstoltzf@jhsph.edu.

Received July 28, 1997.

Accepted for publication January 9, 1998.

The American Society for Clinical Nutrition Inc. does not endorse any commercial enterprise.

152

trichiura infection (10), but probably becomes significant only in heavy infections (11); additionally, the importance of trichuriasis as a cause of iron deficiency in populations is questionable (12, 13). Ascariasis is associated with a small deficit in hemoglobin in some populations (6, 14), for reasons that are not well understood.

This paper reports the effects of the Zanzibar school-based deworming program on the iron status of children in primary school. The program was implemented by the local Ministry of Health and school personnel and consisted of a single 500-mg dose of generic mebendazole as an anthelmintic treatment. The evaluation measured effects on helminth infections, growth, iron status, and school attendance of children receiving either twice-yearly or thrice-yearly deworming. Effects on growth are reported elsewhere (15).

METHODS

The school-based deworming program

School-based strategies for parasite control were begun in Zanzibar in 1988, with a test-and-treat program for control of urinary schistosomiasis (*Schistosoma haematobium* infection). From 1988 to 1992, all children in primary school were tested annually for microhematuria with Hemastix test strips (Ames Laboratories, Elkhart, IN). Those who tested positive were treated with praziquantel. This program effectively controlled urinary schistosomiasis (16), but from 1992 to 1994 the program was temporarily discontinued as a result of a lack of funds. The school-based deworming program that we evaluated began in 1994 on Pemba Island, the smaller of the two islands of Zanzibar, and included a test-and-treat component for urinary schistosomiasis and mass treatment for geohelminths. All program activities were implemented by the Pemba Island Helminth Control Team, a unit of the local Ministry of Health, in cooperation with the Ministry of Education.

A single dose of mebendazole was chosen as the anthelmintic treatment on the basis of both cost and efficacy. A previous randomized trial conducted in Pemba had compared the efficacy of a single dose of 500 mg generic mebendazole with a single dose of 400 mg albendazole. Compared with that of albendazole, the efficacy of mebendazole was somewhat less against hookworms, very similar against *Ascaris lumbricoides*, and somewhat better against *T. trichiura* (17). The cost of generic mebendazole, US$0.027 per dose, was one-tenth of that of albendazole at that time (L Savioli, personal communication, 1993). Because children became rapidly reinfected with all three helminths (18), annual treatment was inadequate.

Program evaluation

Although improved sanitation practices in Zanzibar are a goal of the Ministry of Health, anthelmintic therapy will be the mainstay of helminth control in the coming decade. Given the scarcity of resources for public health programs, evidence of program effectiveness was considered essential for the Ministry of Health to garner sustained program support. A program evaluation that included nonprogram schools was therefore justified and could be done because the program was designed to be implemented over a 2-y period. In addition, a twice-yearly deworming program was implemented in some evaluation schools and a thrice-yearly deworming regimen in others to evaluate the relative effectiveness of the two regimens.

Twelve evaluation schools were selected from the 72 public schools on Pemba Island. From each of the 4 districts on the island, 3 schools were randomly selected and then allocated to the nonprogram, twice-yearly deworming, and thrice-yearly deworming program groups. The same deworming treatment was administered to all children in the selected schools. Placebos were not given in the nonprogram schools, and children, teachers, and investigators were not blinded to the programs being administered. In the twice-yearly deworming group, mebendazole was given in March-April and October-November 1994. In the thrice-yearly deworming group, mebendazole was given in March-April, August, and December 1994. The evaluation was planned and the schools selected before the program was implemented in any school on Pemba Island. The nonprogram schools became program schools in 1995.

Only morning classes of children were assessed in the evaluation so that laboratory work could be completed in the afternoon. Children in grades 1–4 were eligible for the evaluation, but grades 1 and 2 were deliberately oversampled because we expected the program to have the greatest effect on nutritional status in younger children.

Parent meetings were held at each school to provide information about the deworming regimen to be implemented in the school in either 1994 or 1995, the purpose of the evaluation, risks and benefits of children's participation, and alternatives to participation. Anthelmintic drugs were available in local pharmacies to children in all three program groups. The study was approved by the ethical review committees of The Johns Hopkins School of Public Health, the World Health Organization, and the Ministry of Health of Zanzibar.

We estimated that 1000 children per group would be sufficient to detect a difference in mean within-individual changes in hemoglobin of 5 g/L. This estimate accounted for the design effect of randomizing at the school level and allowed for subgroup analyses. The evaluation was designed to compare the effects of twice-yearly and thrice-yearly deworming with no deworming. The sample size was not sufficient for testing differences between the two deworming regimens.

According to the teachers' rosters of classes selected for the survey, 3959 children were enrolled and therefore eligible to participate in the evaluation. The baseline survey conducted in March-May 1994 included 3605 children, 91.1% of those eligible. Children with hemoglobin <70 g/L (n = 125, or 3.5% of the sample) were treated with mebendazole and oral iron and were excluded from these analyses. The deworming regimens were begun at the time of the baseline survey in the program schools. Six-month and 12-mo follow-up assessments were conducted in October-November 1994 and March-May 1995, respectively. Eighty-four percent of children (n = 3028) completed the 12-mo follow-up. The losses to follow-up in each program group are described elsewhere (15).

Nutritional and parasitologic assessments

Iron status was assessed by measuring hemoglobin, erythrocyte protoporphyrin, and serum ferritin in a venous blood sample at baseline and 12 mo. At the 6-mo follow-up, only hemoglobin and erythrocyte protoporphyrin were measured in a capillary sample. We chose these 3 measures because they provide complementary information across the range of iron statuses and because they were feasible in this research setting. Although falciparum malaria is highly endemic in Zanzibar,

these indicators performed reliably in this population (19). Hemoglobin and protoporphyrin were measured directly in a drop of whole blood at the school by using a HemoCue hemoglobinometer (HemoCue AB, Angelhome, Sweden) and a fluorometer (Aviv Biomedical, Lakewood, NJ), respectively. At baseline and 12 mo, the remaining blood was allowed to clot at a cool temperature for ≥30 min and then centrifuged at 1000 × *g* for 20 min at room temperature. Serum samples were stored at −20°C for up to 10 wk, transported to Baltimore in liquid nitrogen, and stored at −70°C until analyzed. Ferritin was measured by using a fluorescence-linked immunoassay (DELFIA system; Wallac Inc, Gaithersburg, MD). The children's weights and heights were measured by use of standard methods (20).

Dietary intakes of the children were not ascertained because dietary interviews could not be conducted reliably with young schoolchildren in the absence of their parents. However, the iron bioavailability of the Pembian diet is certainly low. Cassava is the primary staple food, being the least expensive choice and the easiest to raise in home gardens. Maize, rice, plantains, and breadfruit are added to the diet if affordable. These foods are typically eaten with curries or stews made of legumes, vegetables, and small fish. Larger fish and other seafoods are consumed in small amounts occasionally. Meat is a luxury item not regularly consumed. Bananas and papaya are available throughout the year, whereas mangoes, citrus fruit, and other fruit are available seasonally.

Fecal samples were collected from ≈95% and urine samples from 100% of children surveyed. Blood films were stained with Giemsa stain and malarial parasites were counted against leukocytes, helminth fecal egg counts were determined by the Kato-Katz method, and microhematuria, an indication of urinary schistosomiasis, was determined by using Hemastix (Ames Laboratories, Elkhart, IN). These standard parasitologic methods are described elsewhere (6, 19).

Data analysis

Stunting was defined as a height-for-age *z* score < −2 on the basis of the National Center for Health Statistics and World Health Organization (WHO) reference data (21). Anemia was defined as a hemoglobin concentration < 110 g/L. This is lower than the WHO recommended definition for this age group of 120 g/L (22); however, a 10-g/L lower cutoff screens more efficiently for anemia in blacks (23). Hemoglobin cutoffs <90 and <70 g/L were used to define moderate-to-severe anemia and severe anemia, respectively. Exhausted iron stores were defined as a serum ferritin concentration < 12 g/L and iron-deficient erythropoiesis was defined as a protoporphyrin concentration > 90 μmol/mol heme.

Children's baseline characteristics in each treatment group were compared by using Student's *t* test for continuous variables and the chi-square test for categorical variables (24). Estimates of program effects and their variances were adjusted for correlations among children within the same school by using generalized estimating equations (25). Because some child characteristics differed significantly between the program groups at baseline, these estimates were adjusted for baseline factors in multiple regression models. Program effects on hemoglobin, protoporphyrin, and ferritin were measured as the difference between the within-child change from baseline to 12 mo in each program group compared with the control group. Program effects on the prevalence of abnormal values for iron-status indicators was measured as the difference between the change in prevalence within each program group from baseline to 12 mo compared with the control group.

We also measured program effects on the incidence of moderate-to-severe anemia. Children who had moderate-to-severe anemia at baseline were excluded from this analysis. The incidence rate was calculated on a person-by-time basis by using the 6-mo intervals from baseline to 6 mo and from 6 to 12 mo as independent intervals. The program effect was measured by the relative risk of moderate-to-severe anemia in program groups compared with the control group. An analogous analysis was carried out on the incidence of severe anemia. Multivariate linear and Poisson regression models were used with the generalized estimating equation approach (25) to account for the clustered randomization.

Finally, we tested for predictors of benefit, that is, baseline characteristics of children that were associated with a greater program effect. To do this, we tested the interaction between program group and baseline child characteristics by using multiple regression. Interactions with *P* values < 0.15 were considered potentially significant, depending on their magnitude and biological significance.

RESULTS

Characteristics of study children

The study sample included about equal numbers of boys and girls (**Table 1**). The children's median age was 10 y. More than two-thirds of children were infected with *A. lumbricoides* and >90% of children were infected with *T. trichiura* or hookworms. Malarial infection and microhematuria were also common. Children were short for their ages; the overall prevalence of stunting was 48.5%. The pattern of growth retardation in this group of children has been described in detail elsewhere (20). The iron status of the children at baseline was poor.

Several characteristics of children in the three program groups differed significantly at baseline (Table 1). The two deworming groups had slightly more trichuriasis and hookworm infection than did the control group. The twice-yearly deworming group had less ascariasis, less microhematuria, and better height-for-age *z* scores than did the other groups. The thrice-yearly deworming group tended to have the worst iron status. These baseline factors were included in multivariate regression models of the effects of the program on iron status and retained if found to confound or modify the estimate of program effects.

Program effects on helminth infections

The effects of twice-yearly and thrice-yearly deworming on helminth infections are reported in greater detail elsewhere (15), but are summarized here because they are essential to the interpretation of the nutrition findings. The program achieved high coverage in the first year, with 90% of children in the schools treated twice yearly and 89% in those treated thrice yearly receiving the full regimen. Both deworming regimens effectively controlled ascariasis. The geometric mean (+1 SD, −1 SD) fecal egg counts per g feces for *A. lumbricoides* at 12 mo were 653 (25, 17367), 54 (2, 1433), and 10 (0, 289) in the control, twice-yearly deworming, and thrice-yearly deworming groups, respectively. The deworming programs did not have a large effect on the prevalence of *T. trichiura* or hookworm infections, which

TABLE 1
Baseline characteristics of the study children by program group

Characteristic	Program group: Control (n = 1002)	Twice-yearly deworming (n = 952)	Thrice-yearly deworming (n = 970)
Male sex (%)	50.7	49.6	49.5
Age (y)	10.5 ± 1.6[1]	10.6 ± 2.6	10.5 ± 1.7
Hookworms			
Infected (%)[2]	91.0	94.4	95.7
Eggs/g feces[2]	321 (37, 2775)[3]	492 (75, 3234)	583 (101, 3362)
Trichuris trichiura			
Infected (%)[4]	94.8	96.5	97.1
Eggs/g feces	527 (82, 3370)	577 (108, 3109)	614 (126, 2998)
Ascaris lumbricoides			
Infected (%)[2]	72.7	66.7	75.8
Eggs/g feces[4]	229 (7, 8066)	152 (4, 6432)	314 (10, 10155)
Malaria parasitemia (%)[2]	57.1	56.8	65.4
Microhematuria (%)[2,5]	30.4	19.9	35.0
Height-for-age z score	−1.93 ± 1.23	−1.78 ± 1.44	−1.97 ± 1.39
Body mass index (kg/m^2)	14.6 ± 1.3	14.7 ± 1.5	14.7 ± 1.3
Hemoglobin (g/L)[2]	106 ± 12	107 ± 13	104 ± 13
Hemoglobin < 110 g/L (%)[2]	60.2	56.3	66.3
Protoporphyrin (μmol/mol heme)[4]	89 (55, 144)	92 (57, 150)	97 (58, 164)
Protoporphrin > 90 μmol/mol heme (%)[2]	44.3	45.0	51.7
Ferritin (μg/L)	14.4 (7.8, 26.6)	14.8 (7.6, 28.7)	14.2 (7.5, 27.1)
Ferritin < 12 μg/L (%)	40.2	38.9	40.9

[1] $\bar{x}$ ± SD.
[2] Group proportions are significantly different, $P < 0.01$.
[3] Geometric $\bar{x}$ (−1 SD, + 1 SD).
[4] Group means are significantly different, $P < 0.05$.
[5] An indicator of urinary schistosomiasis.

remained at 85–97% in all three groups. However, deworming did reduce the intensity of infections with these parasites in a frequency-dependent manner. At 12 mo, the geometric mean fecal egg counts for *T. trichiura* were 788 (96, 6449), 340 (41, 2633), and 147 (19, 1256), and for hookworms were 778 (94, 6728), 329 (41, 2826), and 262 (30, 2044) in the control, twice-yearly deworming, and thrice-yearly deworming groups, respectively. The prevalence of moderate-to-heavy hookworm infections (>2000 hookworm eggs/g feces) at 12 mo were 28%, 14%, and 9%, respectively.

Overall program effect on iron status

Children's hemoglobin concentrations improved significantly from baseline to the 12-mo follow-up survey in all program groups. The average hemoglobin concentration increased by ≈11 g/L and the prevalence of anemia declined by almost one-half. This improvement happened in the second 6-mo period of follow-up. At the 6-mo follow-up survey, the mean (±SD) hemoglobin concentration was 105 ± 15 g/L and the prevalence of anemia was 58.8%, values similar to those found in the baseline survey.

The deworming programs did not significantly improve the mean hemoglobin concentration relative to the control group, nor did they reduce the prevalence of anemia relative to the control group (**Table 2**). However, the thrice-yearly deworming regimen did have a positive effect on iron status. The mean values of protoporphyrin were highly significantly improved and those of ferritin were marginally significantly improved in the thrice-yearly deworming group, and the prevalence of iron-deficient values of both indicators was significantly decreased. Twice-yearly deworming had a marginally significantly positive effect on the serum ferritin concentration, but otherwise had no significant effect on iron status of the entire study cohort.

Although the deworming programs had no overall effect on the prevalence of anemia, the incidence of more severe forms of anemia was lower in the thrice-yearly deworming group (**Table 3**). Severe anemia was reduced by 23% in the twice-yearly deworming group and by 55% in the thrice-yearly deworming group. Although the reduction was large in the thrice-yearly deworming group, the 95% CI included unity.

Predictors of benefit from deworming

No child characteristics that we measured were predictive of biologically significant improvements in hemoglobin concentrations from deworming. Lower baseline hemoglobin concentration, male sex, and age > 10 y were statistically associated with greater increases in hemoglobin concentration (ie, their interaction terms with the program group had P values < 0.15), but in no subgroup was the hemoglobin gain associated with either deworming program ≥3 g/L (data not shown).

However, the intensity of hookworm infection at baseline was predictive of the reduction in incidence of moderate-to-severe anemia (Table 3). In children with < 2000 hookworm eggs/g feces at baseline, nether program had a significant effect. In chil-

155

TABLE 2
Program effects on iron status[1]

Characteristic	Program group: Control	Twice-yearly deworming	Thrice-yearly deworming
12-mo Change[2]			
Hemoglobin (g/L)	11.3 ± 1.7	10.3 ± 1.7	12.7 ± 1.7
Protoporphyrin (μmol/mol heme)	−6 ± 3	−13 ± 3	−24 ± 3[3]
Ferritin (μg/L)	2.8 ± 0.7	4.6 ± 0.7[4]	4.5 ± 0.7[4]
12-mo Change in prevalence (%)[5]			
Hemoglobin < 110 g/L	−31.3 ± 5.3	−24.3 ± 5.3	−33.1 ± 5.3
Protoporphrin > 90 μmol/mol heme	−2.9 ± 2.5	−5.3 ± 2.5	−15.8 ± 2.5[3]
Ferritin < 12 μg/L	−10.3 ± 1.5	−11.1 ± 1.7	−14.2 ± 1.5[6]

[1] $\bar{x}$ ± SE.

[2] Values are within-individual differences for each indicator between baseline and 12 mo, adjusted for iron status, sex, age, hookworm infection, district, and height-for-age at baseline, and accounting for within-school correlations.

[3,4,6] *Significantly different from control:* [3] $P < 0.001$, [4] $P = 0.07$, [6] $P = 0.06$.

[5] Values are changes in prevalence of abnormal values for each indicator from baseline to 12 mo, adjusted for iron status, sex, age, hookworm infection, district, and height-for-age at baseline, and accounting for within-school correlations.

dren with moderate or heavy hookworm infections (≥2000 hookworm eggs/g feces), both deworming programs had a large protective effect. Twice-yearly deworming reduced the incidence of moderate-to-severe anemia by 47% and thrice-yearly deworming reduced the incidence by 57%. Put another way, in the control group, children with heavier hookworm infections at baseline had a more than twofold greater risk of developing moderate-to-severe anemia (incidence of 9.8% compared with 4.7%). Both deworming regimens greatly reduced this excess risk.

A similar pattern of effect modification was seen on the prevalence of iron-deficient erythropoiesis (ie, protoporphyrin > 90 μmol/mol heme) at 12 mo (**Figure 1**). In children without hookworm infection or with light infection, thrice-yearly deworming reduced the adjusted prevalence from 39.2% to 33.9%, but twice-yearly deworming had virtually no effect. In children with moderate-to-heavy hookworm infection, the adjusted prevalences were 53.2%, 46.4%, and 35.8% in the control, twice-yearly deworming, and thrice-yearly deworming groups, respectively. Again, thrice-yearly deworming essentially removed the excess risk of iron-deficient erythropoiesis found in children with heavier hookworm infections.

The children's age and baseline hemoglobin concentration strongly predicted the change in protoporphyrin associated with deworming. Children > 10 y of age had larger decreases in protoporphyrin concentrations associated with deworming than did younger children (12 μmol/mol heme greater in the twice-yearly deworming group and 9 μmol/mol heme greater in the thrice-yearly deworming group; interaction of age and both deworming regimens, $P < 0.025$). The decrease in protoporphyrin was significant for both age groups in the thrice-yearly deworming group but for only older children in the twice-yearly deworming group (data not shown). In the thrice-yearly deworming group, children with an initial hemoglobin concentration of 80 g/L had a sixfold greater reduction in protoporphyrin concentration after deworming than did those with a hemoglobin concentration of 120 g/L (−40.0 compared with −6.1 μmol/mol heme, values for children > 10 y; **Figure 2**). In the thrice-yearly deworming group this interaction was highly significant ($P < 0.005$), but in the twice-yearly deworming group it was not as strong.

The benefit to serum ferritin from deworming occurred only in children who had some iron storage at baseline. Children in whom iron stores were exhausted at baseline (serum ferritin < 12 μg/L) showed no improvements in ferritin concentration associated with deworming, whereas those with some iron stores at baseline had 12-mo increases of 3.1 and 3.4 μg/L over the control group (twice-yearly and thrice-yearly deworming groups, respectively; $P < 0.01$ for both groups). This interaction was highly significant in both deworming groups ($P < 0.001$). In children with baseline iron stores, the improvement in serum ferritin concentration was also modified by the child's height-for-age z score (data not shown). Children who were more stunted accumulated more storage iron in association with deworming.

DISCUSSION

The Zanzibar school-based deworming program significantly reduced the burden of iron deficiency and moderate-to-severe anemia in schoolchildren in its first year of implementation. Thrice-yearly deworming caused improvements in iron status measured both by protoporphyrin, a measure of iron-deficient erythropoiesis, and serum ferritin, a measure of iron stores. The most striking benefit was the prevention of moderate-to-severe anemia in children with heavier hookworm infections at baseline. Thus, deworming had the greatest benefit for children at greatest risk for the morbidity and mortality caused by anemia. This benefit was achieved even though the prevalence of hook-

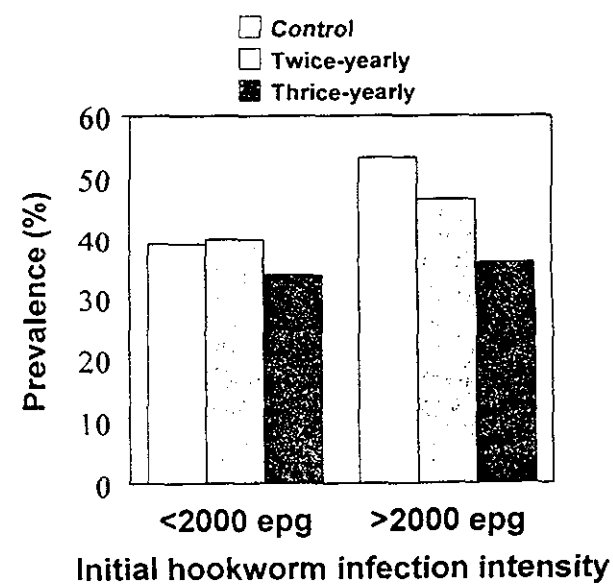


FIGURE 1. Prevalence of iron-deficient erythropoiesis (protoporphyrin > 90 μmol/mol heme) at the 12-mo follow-up in children in the control, twice-yearly deworming, and thrice-yearly deworming program schools stratified by baseline hookworm infection intensity. Epg, eggs/g feces. Values are adjusted for district and for hemoglobin concentration at baseline. Interaction between hookworm infection and twice-yearly deworming, $P = 0.20$; interaction between hookworm infection and thrice-yearly deworming, $P = 0.03$.

156

TABLE 3
Effect of twice-yearly and thrice-yearly deworming on incidence of moderate-to-severe and severe anemia

Severity of anemia and program group	Incidence per 100 intervals[1]	Relative risk[2]	95% CI
Moderate-to-severe anemia (hemoglobin < 90 g/L)			
Control	5.6	1.00	—
Twice-yearly deworming	5.8	1.19	0.72, 1.98
Thrice-yearly deworming	3.5	0.75	0.44, 1.29
Hookworm infection at baseline			
<2000 eggs/g feces			
Control	4.7	1.00	—
Twice-yearly deworming	5.8	1.43	0.87, 2.34
Thrice-yearly deworming	3.2	0.75	0.43, 1.30
≥2000 eggs/g feces			
Control	9.8	1.00	—
Twice-yearly deworming	5.4	0.53	0.29, 0.97
Thrice-yearly deworming	4.7	0.43	0.28, 0.66
Severe anemia (hemoglobin < 70 g/L)			
Control	0.84	1.00	—
Twice-yearly deworming	0.60	0.77	0.39, 1.51
Thrice-yearly deworming	0.35	0.45	0.19, 1.08

[1] Based on estimates from linear regression models, adjusted for hemoglobin, ferritin, and protoporphyrin at baseline.
[2] Based on estimates from Poisson regression models.

worm infection was only modestly reduced, and the intensity of infection was reduced by two-thirds, as measured by fecal egg counts after 1 y.

Despite improvements in iron status, the deworming program had no detectable effects on average hemoglobin concentrations or the prevalence of mild anemia. The differential responsiveness of iron-specific indicators and hemoglobin suggests that factors apart from iron status are important regulators of erythropoiesis in this population and that those other factors were the main causes of the hemoglobin changes we observed. This is corroborated by the fact that hemoglobin concentrations increased dramatically from 1994 to 1995 regardless of treatment (11.3 g/L in the control group), whereas the iron-specific indicators, ferritin and protoporphyrin, changed relatively little in the control group (2.8 μg/L and −6 μmol/mol heme, respectively). Predicting from the empirical relations between hemoglobin and iron-status indicators in our data at baseline (data not shown), the improvements in protoporphyrin and ferritin in the control group would account for a hemoglobin increase of only 0.5–0.6 g/L—about 5% of the increase we observed in the control group.

If not iron status, what then might explain the change in hemoglobin concentration from 1994 to 1995 in these children? Although we did not measure dietary or household food availability, there was anecdotal evidence that 1994 was a lean year, whereas the harvest in 1995 was exceptionally good. Thus, it is possible that other nutritional influences caused by better diet contributed to the increase. The difference in hemoglobin cannot be explained by a systematic error in hemoglobin measurement because the same method was used in all surveys and controls were run daily. Nor did the difference relate to malaria trends because the prevalence and intensity of malaria parasitemia were nearly identical in 1994 and 1995. In sum, the secular change in hemoglobin is perplexing because it is not well explained by the variables we measured and because it appears to have obscured the potential effect on mild anemia that we would expect to be associated with improvements in iron status.

The greater effect of deworming in children with more severe forms of anemia was predicted by the cross-sectional relation between hookworm infection and anemia in these children at baseline. In these analyses, the attributable fraction of anemia associated with hookworm infection was 25%, but the attributable fraction of severe anemia was 73% (6). We expected the actual reduction in anemia and severe anemia achieved from deworming to be less than these attributable fractions because hookworm infection was reduced but not eradicated by periodic deworming.

The pattern of effects that we observed within and between program groups agrees with a continuous relation between the intensity of hookworm infection (ie, the worm burden) and the degree of blood loss. Thrice-yearly deworming reduced hookworm burdens substantially better than did twice-yearly deworming (based on fecal egg counts) and by almost every indicator the effect on iron status was greater in the thrice-yearly deworming group. Furthermore, the effect of deworming on iron-deficient erythropoiesis and moderate-to-severe anemia was significantly greater in children with heavier hookworm infections at baseline. Because even thrice-yearly mebendazole did not reduce the prevalence or intensity of hookworm infection to very low levels, our results suggest that more effective anthelmintic regimens (ie, more frequent administration or more efficacious drugs) would have larger effects on children's iron status.

Anthelmintic therapy might bring about an improvement in children's growth as well as in erythropoiesis and iron storage. These benefits are likely to be in competition, however, because growth is iron costly (26). Previous efficacy trials of deworming of east African schoolchildren (27–31), carried out over periods lasting from 5 wk to 8 mo, found impressive effects on growth but in general did not report impressive effects on iron status. In only one trial of albendazole treatment of hookworm-infected boys was there was a statistically significant effect on hemoglobin of 4 g/L over 4 mo (29).

As predicted by this competition between indicators of iron status and growth, we found that the subgroups of children who benefited most in terms of growth tended to benefit least in terms of iron status. Specifically, age < 10 y predicted greater growth benefit from deworming (15) but predicted less benefit in hemoglobin and protoporphyrin concentrations. Also, the twice-yearly deworming group had the greatest overall weight gain from deworming (15), which possibly explains why the decrease in prevalence of anemia was smallest in this group. Finally, children who were more stunted at baseline benefited least in terms of ponderal and linear growth (15) but accumulated the most storage iron. When evaluating the effect of deworming programs on iron status, it may be necessary to account for the iron costs of growth if a significant growth effect is observed. We did not take this approach in these analyses because the observed growth effect was small (15) and equations for estimating total body iron have not been validated in growing children. The small growth effect that we observed would tend to cause us to underestimate the effect of deworming on iron status.

Benefits to serum ferritin were small, which is not surprising because the population had a high burden of iron deficiency

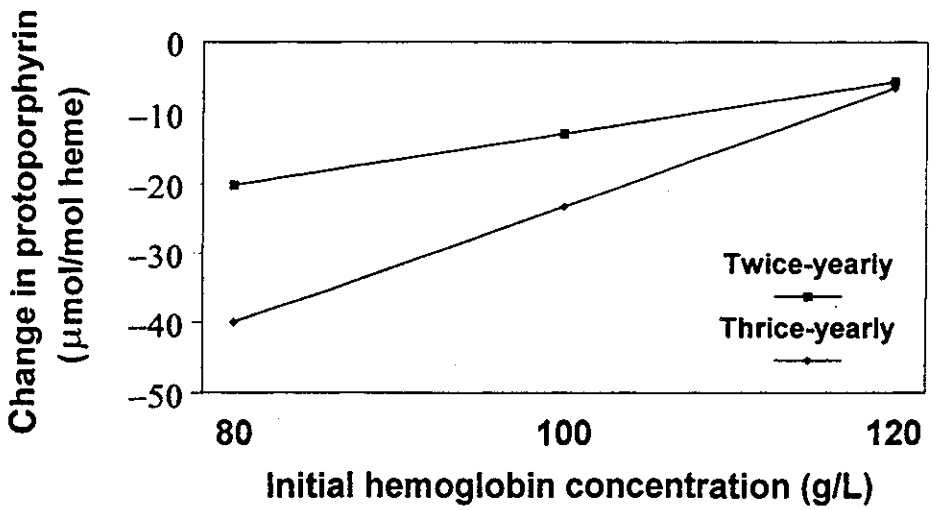


FIGURE 2. Effect of twice-yearly and thrice-yearly deworming on 12-mo change in protoporphyrin concentration according to baseline hemoglobin concentration. Value of zero represents no effect relative to the control group. Values are plotted for children > 10 y of age at baseline, adjusted for district, age, and protoporphyrin and hemoglobin concentrations at baseline. Interaction between hemoglobin and twice-yearly deworming, $P = 0.16$; interaction between hemoglobin and thrice-yearly deworming, $P = 0.001$.

anemia at baseline. The iron saved from reduced intestinal blood loss was utilized for erythropoiesis and growth, not for stores. This explains why the serum ferritin concentration did not increase in children who had no iron stores at baseline.

Because deworming acts primarily by decreasing iron loss, in populations with limited absorbable dietary iron the expected effect from deworming would be to halt or slow the decline in iron status associated with hookworm infection. If the population continues to eat the same poor diet, growing children may not be able to gain body iron, even when hookworm burdens are reduced. Thus, the major effect of anthelmintic therapy in this malnourished population was to prevent children from sliding into moderate or severe iron deficiency anemia. The greatest effects of deworming have been seen in trials in which anthelmintic therapy was combined with increased dietary iron. Significant increases in hemoglobin concentration were reported when deworming was provided along with iron-fortified soup to iron-deficient South African schoolchildren (32), along with iron-fortified salt to an Indian community (8), or along with nutritionally adequate meals to prisoners in Papua New Guinea (33).

This program was administered and implemented by a local unit of the Ministry of Health in collaboration with schoolteachers. The staff of the Helminth Control Team are high-school-educated government employees. The program was well-received by teachers, parents, and students, as shown by the 90% coverage rate achieved. The generic mebendazole tablet is chewable and orange flavored. It was palatable to the children and produced few and mild side effects (17).

The cost of the program, prorated over 10 y, was estimated to be US$4500 per year to cover a population of 30 000 primary school children (Ministry of Health of Zanzibar, unpublished data, 1993). Based on the data in Table 3, we estimate that in 1 y the program prevented 1208 cases of moderate-to-severe anemia at a cost of US$3.57 per case and 276 cases of severe anemia at a cost of US$16.30 per case. These benefits were achieved in a calendar year when children's hemoglobin concentrations improved dramatically apart from the deworming program, and may underestimate the benefit in a more typical year.

We conclude that where hookworm infections are prevalent and iron intakes are poor, deworming programs can marginally improve the iron status of populations and may substantially reduce the incidence of moderate and severe anemia. Those individuals with moderate or heavy hookworm infections will benefit most. This has implications not only for children but also for women, in whom hookworm infections may also be an important cause of anemia (34, 35).

However, hookworm control is not sufficient as an anemia-control strategy. Where delivery systems exist or can be built, increased iron intake through supplementation, fortification, or improved diet should be combined with deworming programs. The addition of iron supplementation to the school-based deworming program in Zanzibar is being evaluated. Drug costs for the thrice-yearly deworming program are $0.08 per child per year and the costs of weekly or daily iron supplementation in schools are at least as low ($0.02 and $0.08 per child per year, respectively). Additional applied research is needed to develop guidelines for when and how deworming is most cost-effectively integrated with other iron interventions.

REFERENCES

1. DeMaeyer EM, Adiels-Tegman M. The prevalence of anaemia in the world. World Health Stat Q 1985;38:302–16.
2. Warren KS, Bundy DAP, Anderson RM, et al. Helminth infection. In: Jamison DT, Mosley WH, Measham AR, Bobadilla JL, eds. Disease control priorities in developing countries. New York: Oxford University Press, 1993:131–60.
3. Banwell JG, Schad GA. Hookworm. Clin Gastroenterol 1978;7:129–55.
4. Roche M, Layrisse M. The nature and causes of "hookworm anemia." Am J Trop Med Hyg 1966;15:1031–100.

5. Stoltzfus RJ, Albonico M, Chwaya HM, et al. HemoQuant determination of hookworm-related blood loss and its role in iron deficiency in African children. Am J Trop Med Hyg 1996;55:399–404.
6. Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KJ, Albonico M, Savioli L. Epidemiology of iron deficiency anemia in Zanzibari school children: the importance of hookworms. Am J Clin Nutr 1997;65:153–9.
7. DeMaeyer EM, Dallman P, Gurney JM, Hallberg L, Sood SK, Srikantia SG. Preventing and controlling iron deficiency anaemia through primary health care: a guide for health administrators and programme managers. Geneva: World Health Organization, 1989.
8. Working Group on Fortification of Salt with Iron. Use of common salt fortified with iron in the control and prevention of anemia—a collaborative study. Am J Clin Nutr 1982;35:1442–51.
9. World Bank. World development report. Investing in health. New York: Oxford University Press, 1993.
10. Layrisse M, Aparcedo L, Martinez-Torres C, Roche M. Blood loss due to infection with *Trichuris trichiura*. Am J Trop Med Hyg 1967;16:613–9.
11. Stephenson LS. Impact of helminth infections on human nutrition. New York: Taylor & Francis, 1987.
12. Greenberg ER, Cline BL. Is trichuriasis associated with iron deficiency anemia? Am J Trop Med Hyg 1979;28:770–2.
13. Lotero H, Tripathy K, Bolanos O. Gastrointestinal blood loss in *Trichuris* infection. Am J Trop Med Hyg 1974;23:1203–4.
14. Curtale F, Tilden R, Muhilal, Vaidya Y, Pokhrel RP, Guerra R. Intestinal helminths and risk of anaemia among Nepalese children. Panminerva Med 1993;35:159–66.
15. Stoltzfus RJ, Albonico M, Tielsch JM, Chwaya HM, Savioli L. School-based deworming program yields small improvement in growth of Zanzibari school children. J Nutr 1997;127:2187–93.
16. Savioli L. Better health in the developing world: collaboration with WHO. Swiss Pharma 1993;15:33–6.
17. Albonico M, Smith PG, Hall A, Chwaya HM, Alawi KS, Savioli L. A randomized controlled trial comparing mebendazole and albendazole against *Ascaris*, *Trichuris* and hookworm infections. Trans R Soc Trop Med Hyg 1994;88:585–9.
18. Albonico M, Smith PG, Ercole E, et al. Rate of reinfection with intestinal nematodes after treatment of children with mebendazole or albendazole in a highly endemic area. Trans R Soc Trop Med Hyg 1995;89:538–41.
19. Stoltzfus RJ, Chwaya HM, Albonico M, Schulze K, Savioli L, Tielsch J. Serum ferritin, erythrocyte protoporphyrin and hemoglobin are valid indicators of iron status of school children in a malaria-holoendemic population. J Nutr 1997;127:293–8.
20. Stoltzfus RJ, Albonico M, Tielsch JM, Chwaya HM, Savioli L. Linear growth retardation in Zanzibari school children. J Nutr 1997; 127:1099–105.
21. World Health Organization. Measuring change in nutrition status: guidelines for assessing the nutritional impact of supplementary feeding programmes. Geneva: World Health Organization, 1983.
22. World Health Organization. Nutritional anaemias: report of a WHO Scientific Group. Geneva: World Health Organization, 1968.
23. Perry GS, Byers T, Yip R, Margen S. Iron nutrition does not account for the hemoglobin differences between blacks and whites. J Nutr 1992;122:1417–24.
24. Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames: Iowa State University Press, 1980.
25. Diggle PJ, Liang KY, Zeger SL. Analysis of longitudinal data. Oxford, United Kingdom: Clavendon Press, 1994.
26. FAO/WHO. Requirements of vitamin A, iron, folate, and B12. FAO Food Nutr Ser 1988;23.
27. Stephenson LS, Kinoti SN, Latham MC, Kurz KM, Kyobe J. Single dose metrifonate or praziquantel treatment in Kenyan children. I. Effects on *Schistosoma haematobium*, hookworm, hemoglobin levels, splenomegaly, and hepatomegaly. Am J Trop Med Hyg 1989;41:436–44.
28. Stephenson LS, Latham MC, Kurz KM, Kinoti SN, Oduori ML, Crompton DWT. Relationships of *Schistosoma haematobium*, hookworm and malarial infections and metrifonate treatment to hemoglobin level in Kenyan school children. Am J Trop Med Hyg 1985;34:519–28.
29. Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A. Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. J Nutr 1993;123:1036–46.
30. Stephenson LC, Latham MC, Adams EJ, Kinoti SN, Pertet A. Weight gain of Kenyan school children infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* is improved following once- or twice-yearly treatment with albendazole. J Nutr 1993;123:656–65.
31. Latham MC, Stephenson LC, Kurz KM, Kinoti SN. Metrifonate or praziquantel treatment improves physical fitness and appetite of Kenyan schoolboys with *Schistosoma haematobium* and hookworm infections. Am J Trop Med Hyg 1990;43:170–9.
32. Kruger M, Badenhorst CJ, Mansvelt EPG, Laubscher JA, Benade AJS. Effects of iron fortification in a school feeding scheme and anthelmintic therapy on the iron status and growth of six-to eight-year-old schoolchildren. Food Nutr Bull 1996;17:11–21.
33. Shield JM, Vaterlaws AL, Kimber RJ, et al. The relationship of hookworm infection, anaemia and iron status in a Papua New Guinea highland population and the response to treatment with iron and mebendazole. P N G Med J 1981;24:19–34.
34. Dreyfuss ML, Shrestha JB, Khatry SK, et al. Relationship between iron status and helminth infection among pregnant women in Nepal. FASEB J 1996;10:A730 (abstr).
35. Stoltzfus RJ, Dreyfuss ML, Chwaya HM, Albonico M. Hookworm control as a strategy to prevent iron deficiency. Nutr Rev 1997; 55:223–32.

Plausible evidence of effectiveness of an iron-supplementation programme for pregnant and post-partum women in rural Bangladesh

Rebecca J. Stoltzfus, Jyatsnamoy Chakraborty, Amy Rice, Bénédicte de la Brière, and Andres de Francisco

Abstract

Since 1978 iron and folic acid pills have been distributed, along with other family-planning and health services, to women in Matlab subdistrict, Bangladesh, through fortnightly visits of community health workers. In 1986 the iron dose was set at three pills daily (198 mg of iron) during pregnancy and two pills daily for 6 months post-partum. Haemoglobin concentrations were determined in a representative sample of 218 women who gave birth from June through August 1994 and who were followed prospectively from 0.5 to 9 months post-partum. At 0.5 months post-partum, 88% of women reported that they had taken iron pills on the previous day. The prevalence of anaemia declined from 36% at 0.5 months to 9% at 9 months post-partum, with an overall average prevalence of 23% in this period. To estimate the impact of the programme, these results were compared to anaemia results from representative samples of non-pregnant women in Matlab in 1975 and 1976 and in three other rural communities in Bangladesh in 1996. The estimated reduction in the prevalence of anaemia resulting from the programme ranged from 48% to 70%, and the estimated increase in haemoglobin concentration ranged from 0.9 to 2.1 g/dl. This evidence suggests that the Matlab programme has been highly effective in controlling anaemia.

Introduction

Iron-deficiency anaemia is the most prevalent form of malnutrition, affecting around 50% of pregnant women worldwide [1]. Iron supplements are inexpensive, widely available, and efficacious, and iron-supplementation programmes have been carried out in many places throughout the world over the last two decades. Still, the prevalence of iron-deficiency anaemia does not appear to be declining [2, 3]. Although most large-scale programmes have not been evaluated, several evaluations have found programmes to be ineffective [4], with no significant decrease in the prevalence of anaemia in the population. To our knowledge, there have been no published reports providing evidence of the effectiveness of a sustained, large-scale iron-supplementation programme.

Although iron-deficiency anaemia is prevalent in many parts of the world, the burden of anaemia in women is highest in South Asia, including Pakistan, India, Nepal, and Bangladesh. Worldwide there are around 420 million anaemic women, of whom 335 million (80%) live in Asia and 160 million (38%) live in South Asia [1]. Clearly, to make substantial progress against the global burden of anaemia in women, effective programmes are needed in South Asia.

We present evidence suggesting that a community-based health-care programme that delivers iron supplements to women during pregnancy and the post-partum period in rural Bangladesh has been highly effective in reducing anaemia in women. Haemoglobin and supplemental iron consumption data were collected as part of a study of vitamin A supplementation and breastfeeding. This provided an opportunity to estimate the impact of an iron-supplementation programme that has been operating for two decades without any evaluation. Because these data were not collected with the intent of evaluating the iron-supplementation programme, the need for additional evaluation data from this programme is highlighted. The elements of the programme that might be keys to its success are discussed.

Rebecca Stoltzfus and Amy Rice are affiliated with the Center for Human Nutrition in the Department of International Health at the Johns Hopkins School of Public Health in Baltimore, Maryland, USA. Jyatsnamoy Chakraborty and Andres de Francisco are affiliated with the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) in Dhaka, Bangladesh. Bénédicte de la Brière is affiliated with the International Food Policy Research Institute in Washington, DC.

160

Methods

Description of the Matlab iron-supplementation programme

This iron-supplementation programme is part of the community-based Maternal Child Health Family Planning Project (MCH-FP) of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Since 1966 ICDDR,B has operated a demographic surveillance system in Matlab thana (subdistrict). The surveillance system provides continuous recording of all births, deaths, migrations, and changes in marital status in a population of 210,000. In 1977 the MCH-FP programme was implemented in half of the surveillance area. In the other half, the health services were provided by the regular government programme. The focus of the MCH-FP programme was initially family planning, but in 1986 it was expanded to include interventions more clearly directed at maternal and child health.

Matlab thana is located 45 km south-east of Dhaka, in a wet and fertile delta area that is interlaced with small rivers and irrigation canals. Rice, jute, and fish are the main agricultural products. The majority of the population are Muslim, but a significant minority (12%) are Hindu. Even though current governmental policies have improved female literacy, most women are functionally illiterate, and about half of adult women have never attended school.

The health services provided by ICDDR,B consist of a central clinic and four subcentre clinics that provide basic medical care. Through the MCH-FP programme, there has also been a system of community health workers who provide primary health-care and basic family-planning services at the household level. All households with a married woman of child-bearing age are visited fortnightly by one of 80 community health workers. These women provide family-planning services, immunizations, breastfeeding promotion, oral rehydration salts for diarrhoea, vitamin A capsules for children under five years of age, and prenatal care, including iron supplements and safe delivery kits, and they are trained to screen people for a variety of health conditions and refer them to health-care facilities. As part of the demographic surveillance system, these health workers also ascertain reproductive status and vital events at each visit.

The community health workers are educated young housewives recruited from the community where they are assigned to work. They are paid staff of the ICDDR,B. After recruitment they are given one month of training, followed by supervisory meetings every two weeks. These meetings focus on the problems the community health workers face daily. An experienced group of paramedical staff conduct training, supervise the work, and provide necessary backup to the community health workers.

The provision of iron supplements to pregnant women by community health workers began in 1978 and has been ongoing ever since with no formal evaluation [5]. The iron tablets are brown-coloured and contain 200 mg of ferrous fumarate (66 mg elemental iron) and 200 g of folic acid. The tablets are procured from local sources at a cost of US$5 per thousand tablets. Beginning in 1978, women were instructed to take one tablet daily from the time a pregnancy was detected by the health worker until six months post-partum. In 1986 the dose was increased to three tablets daily during pregnancy and two tablets daily post-partum. When a pregnancy is detected, the health worker gives the woman a one-month supply of tablets. At subsequent visits, the health worker reviews that the woman is taking the tablets correctly, but reported compliance or occurrence of side effects is not recorded. Initially, acceptance of the tablets was low, and women were suspicious of taking them and complained of side effects. Over time, the tablets gained popularity. The most commonly reported side effects are black stool, nausea, and constipation.

Data collection

Matlab 1994–95 data

Recent maternal haemoglobin data (hereafter called Matlab 1994–95) were collected as part of a randomized trial of vitamin A supplementation and β-carotene supplementation to women in the Matlab MCH-FP intervention area, the RETIBETA study. The details of the main study have been reported elsewhere [6], but the salient points are also described here. We attempted to enroll in the study all women who delivered infants in the MCH-FP area between June 14 and August 29, 1994, and who could be assessed with their live infants at two weeks post-partum. The age distribution, education, and religion of the 218 women enrolled in the study were very similar to those of all women in the MCH-FP area who delivered infants around that time.

Beginning at two weeks post-partum, women in the study were randomly allocated to receive one of three interventions until nine months post-partum: one 200,000 IU vitamin A capsule, followed by daily placebo capsules; daily 7.5 mg β-carotene capsules; or daily placebo capsules. At 0.5, 3, 6, and 9 months post-partum, a random 50% subsample of the enrolled women was brought to the hospital for a health assessment; 93% of enrolled women completed the nine months of follow-up. Haemoglobin concentration was determined from a venous sample of blood using the HemoCue system (Angelholm, Sweden). A control cuvette was checked daily, and no problems were encountered with the instrument. The women's vitamin A status was also assessed at these times. Vitamin A status improved with vitamin A supplementation, but haemoglobin concentrations and anaemia prevalence rates did not differ

between treatment groups, and there was no evidence of any interactions among either form of vitamin A supplementation, iron supplementation, and haemoglobin concentration (data not shown). Therefore, the three treatment groups are combined in these analyses.

The women's reported consumption of iron tablets was ascertained during a dietary interview that included questions on intakes of all types of supplements. Neither the interviewers nor the mothers were aware of any special interest in the iron-supplementation programme. The interviewers were independent of the community health workers who distributed the tablets. Before starting the 24-hour recall in the dietary interview, the women were asked, "Other than the RETIBETA capsule, did you take any vitamin capsule, pill, or liquid yesterday?" Women who responded yes were then asked, "What kind of capsule, pill, or liquid did you take?" MCH-FP iron-folate tablets were recorded as one possible response to this question. Women were not prompted to recall any specific type of vitamin preparation.

All women gave their informed consent to participate in the study, which was reviewed and approved by the Committee on Human Research of Johns Hopkins University and the Ethical Review Committee of ICDDR,B.

Comparison data

To estimate the impact of the iron-supplementation programme on haemoglobin concentrations of the Matlab study sample, non-intervention data were needed for comparison. Two types of comparison data are presented. A survey of haematocrit values of non-pregnant women in Matlab was used as a historical comparison (hereafter called Matlab 1975–76 data). These data were collected in 1975–76 from a representative sample of 2,445 married women below the age of 50 years [7]. These data allow a pre-post-intervention estimate of programme impact, as the iron-supplementation programme did not begin until 1978.

The second type of comparison data consists of other contemporary data from Bangladesh. These data are from a haemoglobin survey conducted by the International Food Policy Research Institute in 1996 in three rural areas of Bangladesh apart from Matlab (hereafter called IFPRI 1996). In these surveys, haemoglobin concentrations of all women in survey households were determined in a representative sample of households, using the HemoCue method and capillary blood samples. The Manikganj site (Saturia thana) is 90 minutes by car from Dhaka, off the main road running west from Dhaka to Jessore. It is a vegetable-growing region, with intensive agriculture for the markets of Manikganj and Dhaka. The Mymensingh study area (Pakundia, Kishoreganj, and Gaffargaon thanas) is a traditional rice-growing area two hours north of Dhaka. The Jessore site is eight hours south-west of Dhaka, near the Indian border. The economy consists of subsistence agriculture and an active labour market based on fruit trees, date juice, rickshaw pulling, and embroidery. In all three study sites, there is no regular programme to provide iron supplements to pregnant women. However, iron tablets have replaced the "placebo" pills in monthly cycles of oral contraceptives used by 20% of women in these survey areas. Out of 28 pills per packet, 7 pills contain 75 mg of iron as ferrous fumarate. Therefore, this comparison population does not represent a non-intervened population, but one in which iron supplementation reaches many fewer women overall and none during pregnancy, is intermittent (seven days per month), and is of lower dose than the Matlab programme. The efficacy of this one week per month supplementation strategy has not been measured; however, iron supplementation one day per week improved iron status in non-pregnant women in Indonesia [8] and the United States [9].

Data analysis

To compare the Matlab 1994–95 data with those from other studies, we needed a summary figure of haemoglobin concentration in the study sample over the post-partum study period. Because the majority of women contributed two haemoglobin measurements, we took the average of all available haemoglobin measurements for each woman and used this value to obtain overall mean haemoglobin concentration and anaemia prevalence. The two haemoglobin measurements for each woman were taken at random intervals post-partum. Thus this overall average value represents a theoretical midpoint of the period from 0.5 to 9 months post-partum.

The Matlab 1975–76 data consisted of haematocrit rather than haemoglobin values. So that these data could be directly compared with the contemporary haemoglobin data, mean and SD haematocrit values were converted to estimated haemoglobin values using a factor of 0.33 [1]. The criterion for anaemia in non-pregnant women is 12 g/dl haemoglobin or 36% haematocrit [10]. The prevalence of anaemia according to this cut-off was not reported by Huffman et al. [7]. We derived an estimated prevalence of haematocrit values less than 36%, based on the reported mean and SD, assuming a normal distribution of values.

The IFPRI 1996 survey included all women in survey households. Two samples were constructed, designed to be comparable to the Matlab 1975–76 and Matlab 1994–95 samples, respectively. The first sample included all married women below 50 years of age, the sample definition in the Matlab 1975–76 study. For this sample definition, haemoglobin and anaemia values are reported separately for each of the three survey communities, to reveal the variability in anaemia between communities. The second sample included all women who re-

162

ported having given birth within the past nine months. This sample definition approximates the Matlab 1994–95 sample. For this sample, haemoglobin and anaemia values are reported for all three communities combined, as the total number of women meeting this definition was only 62.

To make comparisons between study samples, we calculated 95% confidence intervals around the mean haemoglobin values and the prevalences of anaemia [11]. The programme impact was estimated as the percent reduction in anaemia prevalence in the Matlab 1994–95 sample as compared with either the historical (Matlab 1975–76) or contemporary (IFPRI 1996) samples.

Results

Matlab 1994–95 sample

At all times during the post-partum period, the average haemoglobin concentration was above 12 g/dl (table 1). The haemoglobin concentration improved dramatically from the first six months to the ninth month post-partum, reaching a high of greater than 14 g/dl. The prevalence of moderate anaemia, defined as haemoglobin concentration less than 10 g/dl, was 4.7% in the first month post-partum but declined to nil by nine months post-partum. The percentage of women who reported that they consumed iron tablets was 88% immediately post-partum but declined markedly after six months post-partum, the time at which the community health workers were scheduled to stop distributing the tablets.

Comparability of population samples

A potential flaw in the use of historical control data that span a 20-year interval is the effect of secular trends. In particular, the family-planning programme and other health interventions that have been carried out in Matlab might have led to an improvement in women's nutritional status that is independent of the iron-supplementation programme. However, the differences in heights and weights of women in the Matlab 1994–95 and 1975–76 samples were small (table 2). Although the women in the 1994–95 sample were on the average 1.6 cm taller, the weights of the women in the two samples were nearly identical (0.5 kg difference).

A noteworthy difference between the samples is the age of the women, with the 1975–76 sample including a much greater proportion of women over the age of 30. This difference is explained by the fact that the 1975–76 sample was a representative sample of all non-pregnant women of reproductive age, whereas the 1994–95 sample was a representative sample of all women who had recently delivered an infant. The probability of delivering an infant is higher in women 20 to 30 years old than in older women.

The heights and weights of the women in the IFPRI 1996 sample of women of reproductive age were slightly greater than those of the women in the Matlab 1994–95 sample. The IFPRI 1996 post-partum subsample was similar to the Matlab 1994–95 sample in weight (0.7 kg heavier), height (1.5 cm taller), and percentage of women above 30 years of age (4% difference).

Estimates of programme impact

The average haemoglobin values for women in the Matlab 1994–95 data yielded a population prevalence of 23% for anaemia, whereas the prevalence of anaemia in the Matlab 1975–76 sample was 60% (table 3). In the IFPRI 1996 samples, the prevalence of anaemia was 44% to 59% in non-pregnant married women, and 76% in women in the first nine months post-partum. The estimated impact of the Matlab programme in 1994–95 was a 62% reduction in the prevalence of anaemia, as compared with the historical Matlab data. The estimated impact was a 48% to 61% reduction in the preva-

TABLE 1. Haemoglobin concentration, prevalence of anaemia, and self-reported consumption of iron tablets in Matlab, 1994–95, according to time post-partum

Time post-partum (mo)	N^a	% Prevalence of iron tablet consumption	Hb[b] concentration (g/dl)	% Prevalence of anaemia	
				Hb <12.0 g/dl	Hb <10.0 g/dl
0.5	106	87.7	12.5 ± 1.8[c]	35.8	4.7
3	105	75.2	12.4 ± 1.4	42.9	3.8
6	104	22.1	12.7 ± 1.3	30.8	1.0
9	98	14.3	14.1 ± 1.4	9.2	0

a. Haemoglobin concentration was measured in 218 women; for most women measurements were made at two different times post-partum.
b. Hb, Haemoglobin.
c. Plus-minus values are means ± SD.

TABLE 2. Anthropometric and age characteristics of population samples

Study	N	Mean weight—kg (95% CI)	Mean height—cm (95% CI)	Age > 30 yr—%
Matlab 1994–95	218	40.9 (40.3 to 41.5)	149.5 (148.8 to 150.2)	23
Matlab 1975–76[a]	2,445	40.4 (40.2 to 40.6)	147.9 (147.7 to 148.1)	59
IFPRI 1996 (non-pregnant married women <50 yr)				
Manikganj	278	41.6 (40.8 to 42.3)	149.9 (149.2 to 150.5)	58
Mymensingh	234	41.5 (40.6 to 42.4)	149.7 (149.0 to 150.3)	59
Jessore	277	42.8 (42.0 to 43.5)	150.3 (149.6 to 150.9)	55
IFPRI 1996 (women <9 mo post-partum)				
All communities	62	41.6 (40.4 to 42.8)	151.0 (149.7 to 152.2)	27

Abbreviations: CI, confidence interval; IFPRI, International Food Policy Research Institute.
a. From Huffman et al. [7].

lence of anaemia as compared with contemporary non-pregnant women of reproductive age, and a 70% reduction as compared with contemporary women in the first nine months post-partum. The programme impact on average haemoglobin concentration was an increase of 0.9 to 2.1 g/dl.

Discussion

We have documented a low prevalence of anaemia in women in Matlab during the post-partum period, accompanied by a high self-reported consumption of iron supplements. Both figures are remarkable, given the consistent reports in the literature of prevalent and severe anaemia in South Asian women [1, 3] and the poor coverage of iron supplementation documented in other programmes [4]. The obvious question is, "Can the low rates of anaemia in this population be attributed to the iron-supplementation programme?" Because an evaluation was not designed into the programme, we constructed comparison data from existing anaemia surveys and tried to assess the extent and direction of bias that is inherent in non-randomly allocated comparison groups. We draw the following conclusions from these analyses:

» The current prevalence of anaemia is substantially lower in Matlab today than it was before iron supple-

TABLE 3. Haemoglobin concentration and prevalence of anaemia in non-pregnant women in Matlab 1994–95, Matlab 1975–76, and IFPRI 1996 surveys

Study	N	Hb—g/dl (95% CI)	% Prevalence of anaemia[a] (95% CI)	% Impact of Matlab programme on anaemia[b]
Matlab 1994–95[c]	218	12.9 (12.7 to 13.1)	23 (21 to 25)	
Matlab 1975–76[d]	2,445	11.7 (11.7 to 11.8)	60 (58 to 62)	–62
IFPRI 1996 (non-pregnant married women <50 yr)[e]				
Manikganj	278	12.0 (11.8 to 12.1)	47 (41 to 53)	–51
Mymensingh	234	11.3 (11.1 to 11.5)	59 (52 to 65)	–61
Jessore	277	11.9 (11.8 to 12.1)	44 (38 to 50)	–48
IFPRI 1996 (women <9 mo post-partum)				
All communities	62	10.8 (10.4 to 11.2)	76 (63 to 85)	–70

Abbreviations: CI, confidence interval; Hb, haemoglobin; IFPRI, International Food Policy Research Institute.
a. Defined as Hb<12.0 g/dl.
b. Defined as the ratio of the prevalence of anaemia in Matlab 1994–95 to the prevalence in the comparison sample, subtracted from 100%.
c. Calculated from the average of the haemoglobin values available for each woman, usually two values.
d. Estimated from haematocrit values of Huffman et al. [7]. No iron supplements were provided in the study area.
e. Twenty percent of women reported taking oral contraceptive pills in which 7 of the 28 pills contained 75 mg of iron per pill.

164

mentation began, and it is substantially lower than in similar samples of women in three other rural Bangladeshi communities today.

» Women in the comparison samples consumed no supplemental iron, or much less supplemental iron than contemporary women in Matlab were reported to consume.

» The difference in the prevalence of anaemia between the samples is not attributable to differences in age or in general nutritional status of the women, as reflected by their weights and heights.

» The most plausible explanation at hand is that the low rates of anaemia in Matlab are related to the high consumption of supplemental iron provided by the MCH-FP programme. However, this inference is ad hoc and merits a more rigorous investigation.

Our best estimates of programme impact from these data are a 62% reduction in anaemia based on historical Matlab data and a 70% reduction based on a contemporary sample of women at a similar stage post-partum. To our knowledge, this is the first evidence in the published literature of substantial impact from a long-standing health-care programme that delivers iron supplements to pregnant and post-partum women.

What are the major threats to the validity of these conclusions? To begin with, we have identified two possible sources of bias in the comparisons between these data sets. First are the assumptions we made in estimating the prevalence of anaemia from the Matlab 1975–76 data. We used a conversion factor of 0.33 between haematocrit and haemoglobin. This factor depends on the mean cell haemoglobin concentration [12], which will vary among individuals in any study sample. On a population basis, anaemia prevalence rates based on haematocrit tend to be lower than those based on haemoglobin when "equivalent" cut-offs for the two indicators are based on this factor [13]. This bias would cause us to underestimate programme impact. We also assumed a normal distribution of haematocrit values. Examination of numerous haemoglobin distributions from a variety of iron-deficient populations shows that in general the assumption of normality is a reasonable one [14]. When distributions are not normal, they are skewed to low values. If the Matlab 1975–76 data were skewed to low values, this also would bias downward our estimate of the prevalence of anaemia and cause us to underestimate the programme impact.

Second, the Matlab 1994–95 sample consisted of women in the post-partum period, whereas the Matlab 1975–76 sample consisted of all non-pregnant married women below the age of 50 years. Because of these different sampling strategies, the women in the Matlab 1994–95 sample were younger and earlier in the post-partum period than those in the historical comparison sample. The effect of these biases on comparison of the two Matlab samples is illustrated in the comparison of the two IFPRI 1996 samples (table 2). Women in the post-partum period had significantly lower haemoglobin values than all women of reproductive age, even though they were younger. This is almost certainly because women shortly post-partum are still recovering from the substantial iron costs of pregnancy. In summary, these biases would tend to lead us to underestimate programme impact. No bias that we have identified could spuriously create the greater than 50% reduction in the prevalence of anaemia that we observed.

The preceding discussion relates to biases we can identify in the variables we measured. However, the most important threat to validity lies in differences in the samples that we did not measure and therefore cannot account for. Unmeasured differences between the historical and contemporary Matlab samples might include general socio-economic improvements, such as greater mobility of women, changes in infectious disease patterns, and changes in reproductive patterns, with contemporary women having fewer babies and starting at a later age. Despite economic development, however, there is no evidence that dietary intake has improved over the last three decades in rural Bangladesh. Food-intake data collected by the government of Bangladesh show a decline in food availability per capita, which they attribute to the fact that the rate of population growth is outpacing the rate of increase in food production [15]. This may explain the lack of improvement in women's weights between the Matlab 1975–76 and Matlab 1994–95 samples. Unmeasured differences between the contemporary IFPRI and Matlab samples might include differences in reproductive patterns and safe motherhood practices that could have reduced blood loss at delivery in Matlab. We cannot rule out the influences of these variables, and a more rigorous evaluation should account for them.

Evidence of programme impact would be stronger if it could be demonstrated that the haemoglobin concentrations of women who consumed many pills were higher than those of women who consumed few or no pills. Iron-supplementation trials of pregnant women have shown clearly that the impact on haemoglobin is directly related to the duration of supplementation [16]. According to our 24-hour-recall information, women who reported consuming a pill on the previous day at three, six, and nine months post-partum had anaemia rates 25% to 50% lower than women who did not report consumption, but these differences were not statistically significant. The strength of this evidence is limited by our small sample size. Furthermore, we assessed consumption on only four days in the post-partum period (i.e., the day before each clinic visit), although women received supplements throughout most of pregnancy and up to six months post-partum. Prospective information on pill consumption in larger samples of women should be ascertained in future evaluations of this programme.

The low prevalence of anaemia in Matlab at nine

165

months post-partum (9.2% with haemoglobin levels below 12.0 g/dl, and none with levels below 10.0 g/dl) is particularly noteworthy in a rural South Asian population. The substantial improvement in haemoglobin concentrations of Matlab women during the post-partum period probably reflects the effect of continued iron supplementation during a period that is physiologically favourable to iron status. This is because iron contained in the additional red cell mass needed during pregnancy is made available, and iron requirements are relatively low because menses are suppressed during full lactation and the iron content of breastmilk is low [17]. Furthermore, women have low iron stores following pregnancy, and this facilitates the absorption of iron. Thus women tend to be in positive iron balance (i.e., a state of improving iron status) during the post-partum period, especially when consuming supplements.

Some characteristics of the MCH-FP iron-supplementation programme that might account for its apparent success are generalizable lessons. A review of six large-scale iron-supplementation programmes carried out in 1991 identified several major constraints to programme success. These included "low accessibility and utilization of antenatal care, inefficient supply and distribution of supplement...inadequate training and motivation of first line health workers, insufficient and inappropriate counselling of mothers, and failure of effective screening and referral procedures" [4]. It was revealing that the major constraints highlighted in the review had much more to do with the health-care system and health-care providers than with non-compliant behaviour by the recipients. The system of community health workers in Matlab overcomes these constraints. In the context of this well-functioning distribution system, the compliance with and effectiveness of daily iron supplementation with 198 mg of iron as ferrous fumarate appear to be very high. Nevertheless, even if compliance can be achieved at higher doses of iron, we should not give women three iron pills daily if less iron would be equally effective, since reducing the dose would reduce side effects and increase efficiency by reducing costs and logistical difficulties.

Some aspects of the MCH-FP programme are unique. The health-care system in Matlab coordinates with governmental services, but the system of community health workers is sustained with a high level of donor support and with the supervision of an international research centre. The Matlab programme represents one example of an effective iron-supplementation programme. It remains to be demonstrated under what conditions governmental or non-governmental programmes can achieve similar success.

Several questions are unanswered by this evaluation and should be addressed in a further investigation. How many pills do women in Matlab typically consume during pregnancy and the post-partum period? Do women who take fewer pills have lower haemoglobin concentrations? What is the prevalence of anaemia in a representative sample of non-pregnant women and in pregnant women? What benefits do women and community health workers perceive from the iron-supplementation programme?

In 1986, when the Matlab regimen was revised to three pills daily, the iron dose given in the Matlab programme exceeded the international recommendation, which was 120 mg of iron daily during pregnancy, when anaemia is prevalent [18]. The dose given in Matlab is more than triple the current recommendation of 60 mg [10, 19]. This provides a unique opportunity to evaluate the new recommendation in a programmatic context. Could the observed low rates of mild anaemia be sustained if the dose of iron were reduced to around 60 mg (one pill) daily?

As compared with other forms of micronutrient malnutrition, global progress against iron-deficiency anaemia has been slow, if not invisible. This has led to numerous discussions of what we are doing wrong and what innovations can spur progress [4, 20–22]. To substantially reduce anaemia of pregnancy on a global scale, it is likely that prenatal iron supplementation must be combined with interventions that improve women's iron stores before pregnancy [9]. Nonetheless, the high coverage and apparent effectiveness of the Matlab pre- and post-natal iron-supplementation programme sound a timely note of optimism and a reminder to strive to implement better what we know *can* work, while also striving to discover new and complementary approaches.

Acknowledgements

This work was funded by the United States Agency for International Development through cooperative agreement DAN-5116-1-00-8051-00 with Johns Hopkins University and grant HRN-5110A-00-3006-00 to the International Food Policy Research Institute.

References

1. World Health Organization. The prevalence of anaemia in women: a tabulation of available information. WHO/MCH/MSM/92.2. 2nd ed. Geneva: WHO, 1992.
2. UNICEF. The state of the world's children 1995. Oxford: Oxford University Press, 1995.
3. United Nations Administrative Committee on Coordination/Subcommittee on Nutrition. Second report on the world nutrition situation. Vol. 1. Global and regional results. Geneva: ACC/SCN, 1992.
4. Gillespie S, Kevany J, Mason J. Controlling iron deficiency. United Nations Administrative Committee on Coordination/Subcommittee on Nutrition: Geneva, 1991.
5. Fauveau V, Chakraborty J. Women's health and maternity care in Matlab. In: Fauveau V, ed. Matlab: women, children, and health. ICDDR,B Special Publication No. 35. Dhaka: International Centre for Diarrhoeal Disease Research, Bangladesh, 1994:109–38.
6. Rice AL. Impact of vitamin A and beta-carotene supplementation in lactating women in Bangladesh on biochemical indicators of maternal and infant vitamin A status. Doctoral dissertation, Johns Hopkins University, Baltimore, Md, USA, 1998.
7. Huffman SL, Wolff M, Lowell S. Nutrition and fertility in Bangladesh: nutritional status of nonpregnant women. Am J Clin Nutr 1985;42:725–38.
8. Gross R, Schultink W, Juliawati. Treatment of anaemia with weekly iron supplementation. Lancet 1994;344:821.
9. Viteri FE. Iron supplementation for the control of iron deficiency in populations at risk. Nutr Rev 1997;55: 195–209.
10. World Health Organization/UNICEF/United Nations University. Indicators and strategies for iron deficiency and anaemia programmes. Report of a consultation, Geneva, 6–10 December 1993. Geneva: WHO, 1997.
11. Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames, Ia, USA: Iowa State University Press, 1980.
12. Gibson RS. Principles of nutritional assessment. New York: Oxford University Press, 1990.
13. Graitcer PL, Goldsby JB, Nichaman MZ. Hemoglobins and hematocrits: Are they equally sensitive in detecting anemias? Am J Clin Nutr 1981;34:61–4.
14. Yip R, Stoltzfus RJ, Simmons WK. Assessment of the prevalence and the nature of iron deficiency for populations: the utility of comparing haemoglobin distributions. In: Hallberg L, ed. Iron nutrition in health and disease. London: John Libby, 1996:31–48.
15. Government of Bangladesh. National plan of action for nutrition (NPAN). Dhaka: Government of Bangladesh, 1995.
16. Sloan NL, Jordan EA, Winikoff B. Does iron supplementation make a difference? Working Paper No. 15. Washington, DC: MotherCare/Population Council, 1992.
17. National Research Council. Recommended Dietary Allowances. 10th ed. Washington, DC: National Academy Press, 1989.
18. DeMaeyer EM. Preventing and controlling iron deficiency anaemia through primary health care. A guide for health administrators and programme managers. Geneva: World Health Organization, 1989.
19. Stoltzfus RJ, Dreyfuss ML. Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia. Washington, DC: International Life Sciences Institute, 1998.
20. Galloway R, McGuire J. Special report. Daily versus weekly: How many iron pills do pregnant women need? Nutr Rev 1996;54:318–23.
21. Solomons NW. Daily versus weekly iron: We still might not be asking the right questions. Nutr Rev 1997;55: 141–2.
22. Stoltzfus RJ. Rethinking anaemia surveillance. Lancet 1997;349:1764–6.

American Journal of Epidemiology
Vol. 148, No. 10
Printed in U.S.A.
Copyright © 1998 by The Johns Hopkins University School of Hygiene and Public Health
All rights reserved

Relation of Hemoglobin Measured at Different Times in Pregnancy to Preterm Birth and Low Birth Weight in Shanghai, China

Li-Ming Zhou,[1] Wen-Wei Yang,[2] Jia-Zeng Hua,[2] Chun-Qin Deng,[1] Xuguang Tao,[1] and Rebecca J. Stoltzfus[3]

This paper addresses two questions: 1) What is the relation of hemoglobin in the second gestational month to preterm birth and low birth weight? 2) How does the relation differ when hemoglobin in the fifth or eighth month or the lowest pregnancy hemoglobin are examined in place of first trimester values? These relations were examined prospectively in 829 women from Shanghai, China in 1991–1992. The population was nearly homogeneous by race, parity, antenatal care, and smoking. Rates of birth outcomes were compared between hemoglobin categories based on 10 g/liter groupings, with 110–119 g/liter as the reference group. Rates of low birth weight and preterm birth (but not small-for-gestational age) were related to early pregnancy hemoglobin concentration in a U-shaped manner. The relative risks (95% confidence intervals) for preterm birth in women by g/liter of hemoglobin were 2.52 (0.95–6.64) for ≥130 g/liter, 1.11 (0.41–2.99) for 120–129 g/liter, 1.64 (0.77–3.47) for 100–109 g/liter, 2.63 (1.17–5.90) for 90–99 g/liter, and 3.73 (1.36–10.23) for 60–89 g/liter. Use of hemoglobin values in the fifth or eighth month attenuated the association with preterm birth. When lowest pregnancy hemoglobin values were used, the association of anemia with both outcomes was obscured, and risk of preterm birth at high hemoglobin values increased dramatically. *Am J Epidemiol* 1998;148:998–1006.

anemia; hemoglobins; infant, low birth weight; infant, premature; infant, small for gestational age; pregnancy

The significance of low hemoglobin values during pregnancy for the health of the fetus has been a source of continuing controversy. Because around half of the world's women experience anemia during pregnancy (1), the impact of pregnancy anemia on fetal development is a question of great public health importance. Because of its high prevalence, if pregnancy anemia puts the fetus at even a modest risk of low birth weight, then a significant proportion of the low birth weight in the world could be prevented through anemia prevention. Preventing low birth weight will save child lives, because low birth weight is one of the strongest predictors of infant mortality (2).

This question would be answered best by a randomized trial of iron supplementation. However, few trials have been conducted because it is unusual to find settings where the standard of antenatal care does not include iron supplementation to women who are known to have anemia. Those trials that have been conducted have been too small to generate definitive conclusions, and most have been conducted in populations where iron-deficiency anemia is rare (3, 4).

Several prospective studies have reported an association between pregnancy anemia and low birth weight (5–10), but a causal relation has not been established (4, 11–14). These studies have suffered from several problems. First, in most populations, women who are anemic also have other characteristics that cause low birth weight. In the United States, for example, anemic women are more likely to be African American and poor (6). It is very difficult to control adequately for these differences between anemic and non-anemic women, because our measurement variables do not capture all that it means to be black and poor in the United States.

Second, plasma volume expansion and lowered hemoglobin concentration are a physiologic response to pregnancy (15). Thus, women with mild "pregnancy anemia" may be a combination of those with iron deficiency (possibly associated with poor pregnancy outcome), and healthy women with a large plasma volume expansion (associated with good pregnancy outcome (16, 17)).

Third, investigators of this question have measured hemoglobin at different times in pregnancy. In mid-

Received for publication August 1, 1997, and accepted for publication April 10, 1998.

Abbreviations: CI, confidence interval; RR, relative risk; SD, standard deviation.

[1] Shanghai Medical University, Shanghai, China.
[2] Shanghai Station of Maternal Health, Shanghai, China.
[3] Center for Human Nutrition, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.
Reprint requests to Dr. Rebecca J. Stoltzfus, Center for Human Nutrition, School of Hygiene and Public Health, The Johns Hopkins University, 615 N. Wolfe Street, Baltimore, MD 21205.

168

pregnancy, hemoglobin concentrations drop as plasma volume expansion outpaces expansion of the red cell mass, while in late pregnancy, plasma volume ceases to expand and hemoglobin concentrations rise if iron stores are adequate. These physiologic processes drive the hemoglobin concentration in different directions at different stages of pregnancy, and are also related to birth outcome. Thus, the shape of the relation of hemoglobin concentration to birth outcome will probably depend on when the hemoglobin is measured, although this has not been fully described. A spurious relation between anemia and preterm birth is created when hematocrit values at delivery are used, because of the physiologic rise in hematocrit in the late third trimester (14). Biologic interpretation of associations is especially problematic when investigators relate birth outcome to the lowest measured pregnancy hemoglobin value (9, 10), which might occur at different times in pregnancy in different women and thus reflect different processes.

We report the results of a prospective observational study of pregnancy hemoglobin concentration and low birth weight in Shanghai, China. This population offered a unique opportunity to examine this issue, because the population is relatively homogeneous, anemia is common, and other known risk factors for low birth weight are uncommon. In addition, women report for prenatal care very early in pregnancy, so that hemoglobin concentration could be measured before the plasma volume had expanded significantly. Two major questions are addressed: 1) What is the relation of hemoglobin in the second gestational month to preterm birth and low birth weight? 2) How does the relation differ when hemoglobin in the fifth or eighth month or the lowest pregnancy hemoglobin are examined in place of second month values?

MATERIALS AND METHODS

The study population comprised pregnant women in the urban area of Shanghai, China. Five out of 23 urban districts were randomly selected, and, from each of those districts, two communities were randomly selected as the study communities. By law, every woman who intends to become pregnant must apply for an approval of birth from the local Family Planning Office before becoming pregnant. Women were also required to report to this office as soon as they knew they were pregnant. By reporting their pregnancies, they received free antenatal care and other benefits. Because Chinese women are usually permitted to bear only one child, a great deal of care and attention is given to this pregnancy. From June 1991 to June 1992, all women who reported a pregnancy were eligible for the study, and 900 women were identified and enrolled. Informed consent was obtained from each woman at time of entry into the study.

The study sample comprises 829 women who completed the pregnancy follow-up and had singleton live deliveries. Of the 71 women who were enrolled but who are excluded from this sample, four delivered twins, 15 had induced abortions, eight had spontaneous abortions, 29 moved and could not be followed, and 15 were lost to follow-up. Complete hemoglobin data were obtained from the remaining 829 women.

When women reported to the community health care facility for antenatal care, an enrollment profile was established. The mean (standard deviation (SD)) gestational age at enrollment was 7.1 (0.9) weeks (range: 6.1–8.4 weeks). Gestational age was based on the last menstrual period. Hemoglobin concentrations were measured at enrollment and again in the fifth and eighth months of pregnancy. Treatment for anemia, consisting of vitamin C (500 mg/d), iron sulfate (250 mg/d), and folic acid (5 mg/d), was offered free to women with hemoglobin values <110 g/liter, and vitamin and mineral preparations were also available in shops. Women were asked at each trimester follow-up whether they were taking iron supplements. A positive response to this question included women who expressed willingness to take iron tablets offered to them at that visit.

The height and weight of the women were measured at the enrollment visit, but were not measured thereafter. The infants' weights and lengths were measured at birth. Low birth weight was defined as <2,500 g. Preterm birth was defined as <37 completed weeks of gestation. Small-for-gestational age was defined as birth weight below the sex-specific 10th percentile of weights for gestational age published by Alexander et al. (18) from the 1991 US Live Birth File of the National Center for Health Statistics. Although this reference was not based on Chinese infants, US references are appropriate for defining small-for-gestational age in Chinese populations (19).

To examine the relation between early pregnancy anemia and low birth weight, the cohort of women was divided into six groups based on their first-trimester hemoglobin level. Women with first-trimester hemoglobin concentrations 110–119 g/liter were considered the reference group. The rates of low birth weight, preterm birth, and small-for-gestational age were examined by hemoglobin group, and relative risks (with 95 percent confidence intervals) were calculated for each group relative to the reference group. Other maternal characteristics that might influence birth weight were also examined by hemoglobin group. Multiple logistic regression (20) was used to calculate odds ratios for the three birth outcomes, adjusting for other

169

maternal characteristics. In the logistic model, hemoglobin groups were treated as dummy variables, with 110–119 g/liter as the reference group. Systolic and diastolic blood pressure were also treated as dummy variables with five levels.

The population attributable risk for preterm birth and low birth weight associated with anemia was calculated by the method of Kahn (21). Anemia was defined as hemoglobin <110 g/liter, which is the 5th centile of the first trimester hemoglobin distribution of healthy European women taking iron supplements (22). This was also the hemoglobin concentration below which risk of adverse birth outcomes began to increase.

The relation of early pregnancy hemoglobin to birth outcomes was then compared with that of later hemoglobin measurements and lowest pregnancy hemoglobin using similar methods. Lowest pregnancy hemoglobin was defined as the lowest of the three hemoglobin measurements taken in pregnancy.

RESULTS

Early pregnancy hemoglobin and birth outcomes

At enrollment, the mean (SD) hemoglobin concentration of the sample was 110 (12) g/liter, and the prevalence of anemia (hemoglobin <110 g/liter) was 48.7 percent. As pregnancy progressed, the mean hemoglobin concentration fell about 5 g/liter (table 1), and the prevalence of anemia rose to 66.2 percent in the second trimester and 67.4 percent in the third trimester. The longitudinal trends in hemoglobin concentration stratified by initial hemoglobin group reflect the overall decline in hemoglobin values, combined with a strong tendency for high and low values to regress toward the mean (table 1).

Usage of iron supplements was low, around 9 percent in the first two trimesters, and did not vary greatly by maternal hemoglobin level or stage of pregnancy (table 1). However, 40.6 percent of women with initial hemoglobin values <90 g/liter reported that they were currently taking or would begin to take iron supplements at the enrollment visit, compared with only 8.2 percent of women in the higher hemoglobin groups combined ($p < 0.001$).

Most women were aged 20–30 years (table 2); none was below 20 years of age. Body mass index, a measure of fatness (calculated as weight (kg)/height (m)^2) averaged 20 at enrollment—a value on the thin side of the normal range. Only 4.5 percent of women had given birth previously. On average, women in the study made more than eight visits to the antenatal clinic, approximately one visit per month of pregnancy. Maternal age was significantly and directly associated with hemoglobin level. None of the other variables in table 2 varied significantly with women's hemoglobin level at enrollment, nor did maternal height, weight, or blood pressure (data not shown). Eight women reported smoking at the enrollment visit, but then stopped during the pregnancy. No woman reported consuming alcohol at any pregnancy visit.

The average (SD) birth weight of study infants was 3,267 (457) g, and the overall rates of preterm birth, low birth weight, and small-for-gestational age were 6.9 percent, 4.6 percent, and 11.5 percent, respectively. Mean birth weight declined with descending hemoglobin stratum (table 2), but this trend was not statistically significant ($p = 0.36$). However, the simple linear regression of birth weight on hemoglobin concentration was highly statistically significant ($p < 0.0001$), with each 10 g/liter increment in hemoglobin associated with a 67 g increment in birth weight.

TABLE 1. Mean hemoglobin concentration and percent iron supplement usage during pregnancy, by hemoglobin group, Shanghai, China, 1991–1992

Hemoglobin group* (g/liter)	No.	Hemoglobin concentration (g/liter), by week			Iron supplement usage (%), by week		
		4–8†	16–20	28–32			
		Mean (SD)‡	Mean (SD)	Mean (SD)	4–8	16–20	28–32§
≥130	57	135 (6)	109 (12)	111 (10)	8.8	8.8	5.3
120–129	129	122 (3)	111 (11)	110 (10)	9.3	7.0	13.0
110–119	239	114 (3)	108 (11)	107 (8)	7.1	8.4	11.3
100–109	263	104 (3)	103 (9)	105 (9)	6.5	9.1	16.3
90–99	109	95 (3)	97 (9)	100 (10)	12.8	12.8	14.0
<90	32	82 (6)	97 (13)	99 (14)	40.6	15.6	18.8
All women	829	110 (12)	105 (11)	106 (10)	9.4	9.3	13.4

* Hemoglobin groups are based on initial (4–8 weeks) hemoglobin concentrations.
† Pregnancy weeks.
‡ SD, standard deviation.
§ Chi-square test for trend of iron supplement usage by hemoglobin group, $p = 0.05$.

170

TABLE 2. Maternal characteristics and birth weight, by hemoglobin group, Shanghai, China, 1991–1992

Hemoglobin group* (g/liter)	No.	Age (years)		BMI† (kg/m²)		Multiparity (%)	Antenatal visits		Birth weight (g)	
		Mean	(SD)‡	Mean	(SD)		Mean	(SD)	Mean	(SD)
≥130	57	27.7	(4.7)§	20.1	(2.1)	8.8	8.6	(3.1)	3,364	(514)
120–129	129	26.2	(4.2)	20.1	(2.4)	1.6	8.3	(3.5)	3,275	(437)
110–119	239	25.3	(3.4)	19.9	(2.4)	7.8	8.7	(3.3)	3,284	(453)
100–109	263	25.2	(3.6)	20.0	(2.3)	4.6	8.2	(3.4)	3,240	(436)
90–99	109	25.1	(3.5)	20.4	(2.4)	9.2	8.3	(3.0)	3,265	(488)
<90	32	24.8	(3.0)	20.0	(2.4)	0	8.3	(3.0)	3,166	(505)
All women	829	25.5	(3.8)	20.0	(2.3)	4.5	8.4	(3.3)	3,267	(457)

* Hemoglobin groups are based on initial (4–8 weeks) hemoglobin concentrations.
† Body mass index (BMI) at first report of pregnancy.
‡ SD, standard deviation.
§ Significant decreasing trend in age with descending hemoglobin group, $p < 0.01$.

The rates of preterm birth and low birth weight were related to women's enrollment hemoglobin level in a U-shaped fashion, with the lowest risk found for women in the 110–119 g/liter hemoglobin group (table 3). When the relative risk of preterm birth was calculated separately for enrollment hemoglobin strata, the 95 percent confidence limits did not include unity for hemoglobin groups below 100 g/liter. The relative risk of low birth weight was significantly different from unity for women in hemoglobin groups 100–109 g/liter and 90–99 g/liter. In the <90 g/liter hemoglobin group, the relative risk for low birth weight was large (relative risk (RR) = 2.99), but the confidence interval was wide because of the small sample size. The risks of preterm birth and low birth weight in the highest hemoglobin group (≥130 g/liter) were 2.5 times higher than in the reference group, but the confidence limits included unity. For all anemic women combined, the risk of preterm birth was doubled and the risk of low birth weight was tripled compared with the reference group. In contrast, the rate of small-for-gestational age did not vary significantly across hemoglobin groups.

Multivariate logistic regression models were constructed to estimate the relative risk of preterm and low birth weight associated with hemoglobin group adjusted for other maternal characteristics. Variables entered into the model were maternal age, gravidity, parity, height, weight, body mass index, blood pressure, and infant sex. With low birth weight as the outcome variable, no additional variables remained statistically significant in the model. The expected relation between body mass index and both small-for-gestational age and low birth weight (11) was apparent in these data, but was not statistically significant because of the small variance in body mass index (not shown). With preterm birth as the outcome, having had a previous pregnancy was found to be significantly protective. The adjusted relative risks for preterm birth for each hemoglobin group were within 10 percent of the unadjusted relative risks presented in table 3, with no meaningful changes in the confidence intervals.

Based on the relative risks observed in all anemic women and the prevalence of anemia in the second month of pregnancy, anemia in early pregnancy accounted for 25 percent of preterm births and 33 percent of low birth weight in this population. These

TABLE 3. Relative risks (RR) of preterm birth, low birth weight, and small-for-gestational age by hemoglobin group, Shanghai, China, 1991–1992

Hemoglobin group* (g/liter)	No.	Preterm			Low birth weight			Small-for-gestational age		
		No. of cases	RR	95% CI†	No. of cases	RR	95% CI	No. of cases	RR	95% CI
≥130	57	6	2.52	0.95–6.64	3	2.52	0.62–6.28	5	0.75	0.30–1.85
120–129	129	6	1.11	0.41–2.99	5	1.85	0.55–6.28	15	0.99	0.55–1.79
110–119	239	10	1.00		5	1.00		28	1.00	
100–109	263	18	1.64	0.77–3.47	15	2.73	1.01–7.39	31	1.01	0.62–1.63
90–99	109	12	2.63	1.17–5.90	8	3.27	1.09–9.77	13	1.02	0.55–1.89
<90	32	5	3.73	1.36–10.23	2	2.99	0.60–14.76	3	0.80	0.26–2.48
<110	404	35	2.07	1.04–4.10	25	2.96	1.15–7.62	45	0.99	0.64–1.54

* Hemoglobin groups are based on initial (4–8 weeks) hemoglobin concentrations.
† CI, confidence interval.

171

values may be interpreted as the maximal proportions of preterm birth and low birth weight that could be prevented if early pregnancy anemia was eradicated in this population (21).

Relation of other hemoglobin measurements to low birth weight and preterm birth

As pregnancy progressed, the hemoglobin distribution of the study cohort shifted toward lower values (figure 1). The distributions in the fifth and eighth months of pregnancy were very similar to each other, with distinct modes at 100–109 g/liter and less spread than in the second month. The distribution of lowest pregnancy hemoglobin had more values in the lowest category (<90 g/liter, 18.5 percent of values) than in the normal range (≥110 g/liter, 15.0 percent of values).

The relation of preterm birth to second and third trimester hemoglobin concentrations had a U-shape similar to the curve for first trimester values (figure 2). However, the curves were somewhat attenuated, especially with second trimester values. None of the relative risks for any hemoglobin strata defined by second or third trimester values differed significantly from unity (data not shown). When the lowest pregnancy hemoglobin value was used, the risk of preterm birth was not as high at lower hemoglobin values compared with the first trimester curve, although the risk was much higher at high hemoglobin values. Using lowest pregnancy hemoglobin value, the relative risk (95 percent confidence interval (CI)) of preterm birth was 4.90 (1.33–18.07) in women with values ≥120 g/liter compared with women with values 110–119 g/liter.

The relation of low birth weight to second and third trimester hemoglobin values was nearly identical to the curve based on first trimester values (figure 3). The relative risks for hemoglobin strata using later pregnancy values were very similar in magnitude and statistical significance to those based on first trimester values (data not shown). The relation of low birth weight to lowest pregnancy hemoglobin differed from the curves based on specific gestational times. The point of minimal risk was 10 g/liter lower using lowest hemoglobin values compared with first trimester values, and the relative risks were lower at both ends of the curve. Using lowest hemoglobin values, the relative risk of low birth weight with 110–119 g/liter as

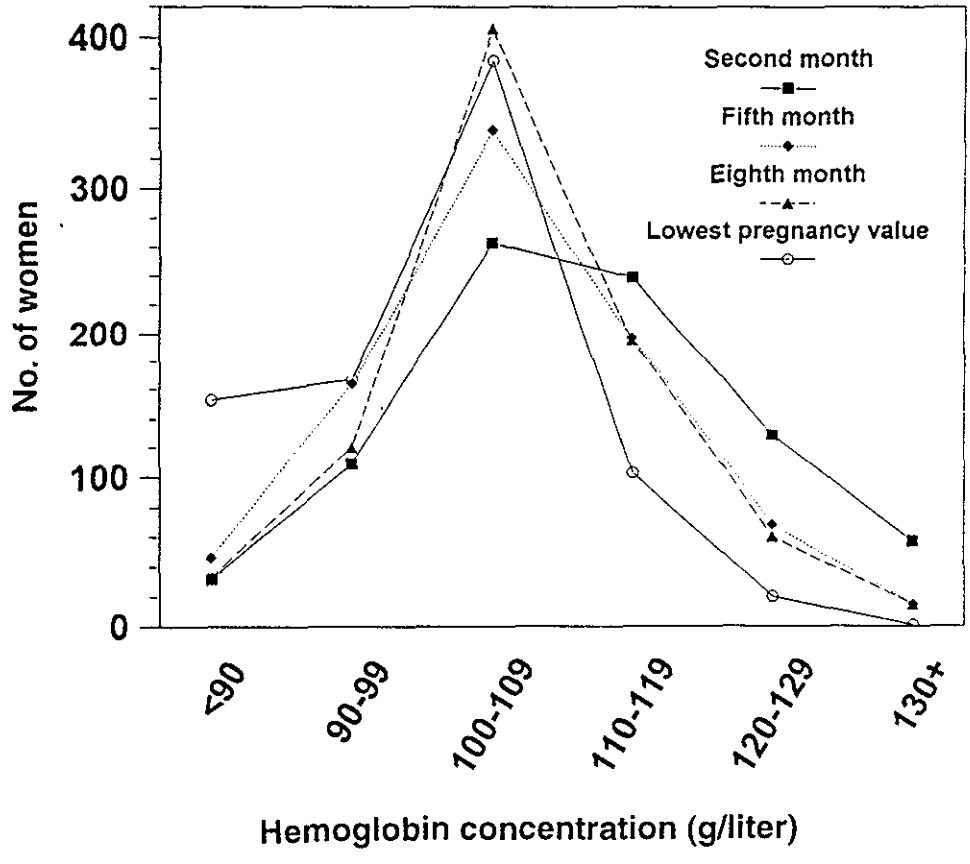


FIGURE 1. Distribution of hemoglobin values in the second, fifth, and eighth months of pregnancy and lowest pregnancy values: Shanghai, China, 1991–1992.

172

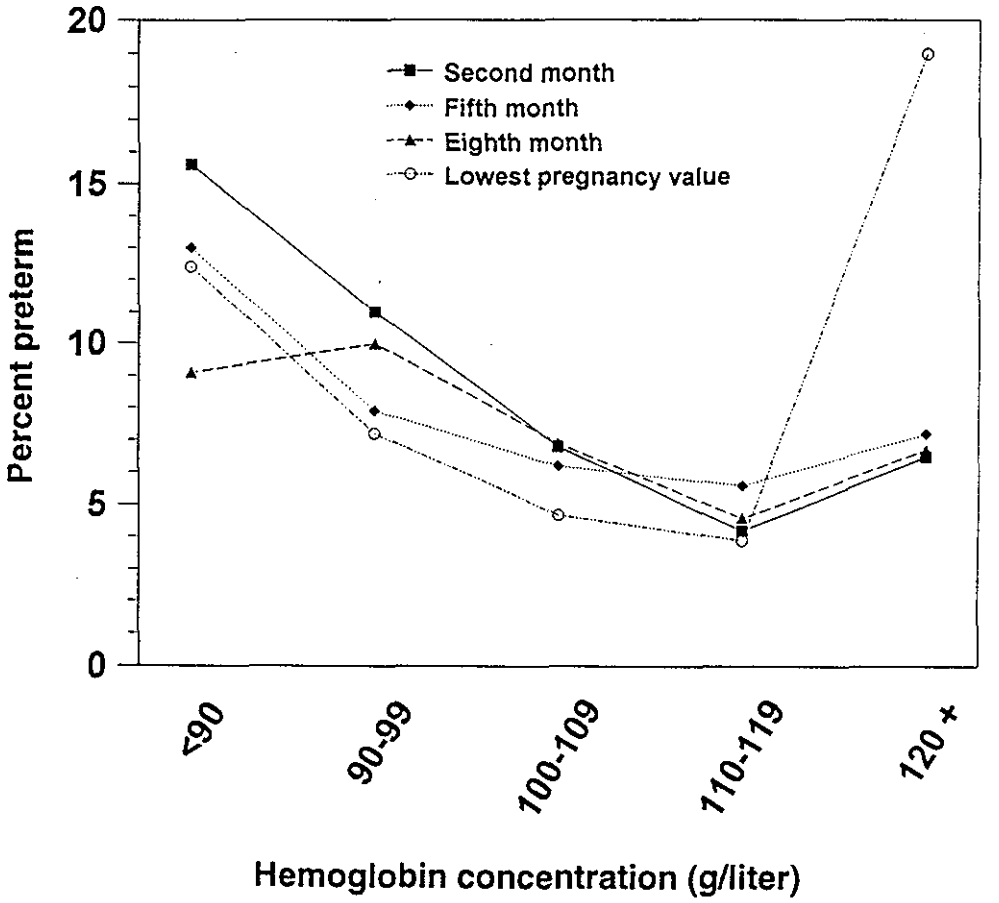


FIGURE 2. Rates of preterm birth by hemoglobin strata based on measurements in the second, fifth, or eighth month of pregnancy or the lowest pregnancy hemoglobin: Shanghai, China, 1991–1992.

reference group was small (ranging from 0.94–1.29 with 95 percent CIs including unity) except in women with values <90 g/liter (RR = 1.85, 95 percent CI 0.61–5.66).

None of the hemoglobin measures, whether taken in the second or third trimester of pregnancy or the lowest pregnancy value, was significantly related to small-for-gestational age.

DISCUSSION

We have observed a strong relation between early pregnancy anemia and risk of low birth weight that derives from preterm birth and cannot be explained by other known risk factors. In previous observational studies, pregnancy anemia was associated with race, smoking, or low prepregnancy body mass index (6–10). These factors are independently associated with low birth weight (11, 12), and therefore might be the true explanation for the observed association between anemia and low birth weight. In the present study, anemia was not associated with these other risk factors. Pregnancy weight gain was not measured in this study, but is not a risk factor for preterm birth (13), so unmeasured differences in pregnancy weight gain cannot explain our findings.

In China, known risk factors for low birth weight are relatively uncommon (19). In part, this is because of the very high value placed on the health of the fetus, combined with good access to antenatal care. The mean birth weight of 3,267 g in this sample of women is similar to other published data from Chinese populations (23–27). The rate of low birth weight (4.6 percent) is very close to the rate observed in a previous study of birth weight in Shanghai (4.4 percent (27)), and slightly below the surveillance rates for China reported by the World Health Organization (6.0 percent (24)). The good health characteristics of pregnant Chinese women explain the low incidence of low birth weight consistently observed in Chinese populations (23). The intensive prenatal care system explains the high follow-up rates achieved in this study.

Anemia, however, remains common among Chinese women, who consume a diet low in heme sources of iron and vitamin C (28). In some parts of China,

173

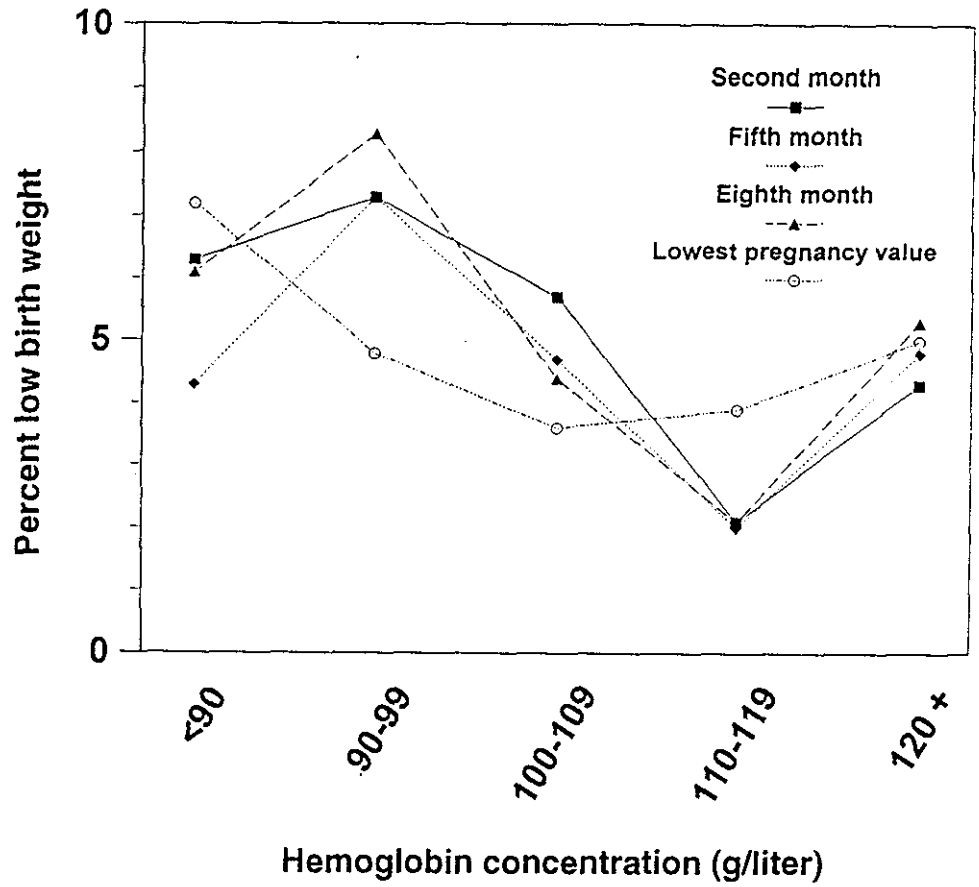


FIGURE 3. Rates of low birth weight by hemoglobin strata based on measurements in the second, fifth, or eighth month of pregnancy or the lowest pregnancy hemoglobin: Shanghai, China, 1991–1992.

hookworm infection is prevalent and likely contributes to the prevalence of anemia, but this is not true of urban Shanghai (29). The fact that hemoglobin values did not improve from the fifth to eighth month of pregnancy is evidence that the women's iron stores were not sufficient to continue the expansion of red cell mass in the third trimester of pregnancy. If iron stores are adequate, hemoglobin concentrations rise from the second to the third trimester of pregnancy (22). Although inflammation from other chronic and infectious disease causes a proportion of anemia in this and other settings (30), given the high prevalence of anemia observed, the most important cause of anemia was undoubtedly dietary iron deficiency.

The observed relation between early pregnancy hemoglobin concentration and birth outcomes cannot be explained by variation in the maternal plasma volume response to pregnancy. At 7 weeks gestation, the average time of the enrollment visit in this study, the hemoglobin concentration has fallen 4 g/liter (95 percent CI 2.3–5.5) from its pre-pregnancy level (15), mostly due to an increase in plasma volume. This change is very small compared with the 30 g/liter range in initial hemoglobin concentration over which we observed a dose-dependent relation with preterm birth. To the extent that women's initial hemoglobin levels were influenced by the size of their plasma volume response, the association between lower hemoglobin concentration and adverse birth outcomes would be attenuated. This would lead to an underestimation of the true role of iron deficiency anemia as a cause of low birth weight.

Despite the fact that the first trimester hemoglobin concentrations were measured before the plasma volume has expanded much, the risks of preterm birth and low birth weight were higher in women with relatively high hemoglobin levels in early pregnancy. The relative risk of 2.5 that we observed for both preterm birth and low birth weight is biologically significant, although it did not reach statistical significance in our small sample. An increased risk of preterm and low birth weight with high maternal hemoglobin has also been observed in previous observational studies (5, 8–10). Since hemoglobin concentrations were measured later in pregnancy in those studies, previous findings could be explained by poor plasma volume

174

expansion in women with high hemoglobin concentrations (5, 8–10). Our findings cannot be explained in this way, which raises the potentially important question of what underlies high hemoglobin concentration early in pregnancy.

Higher hemoglobin concentrations (i.e., >120 g/liter) around the 10th week of pregnancy were weakly correlated with lower concentrations of chorionic gonadotropin and placental lactogen concentrations in British women (31). From those findings, Wheeler et al. (31) speculated that when the oxygen content of blood is quite high, early placental development is adversely affected. Fleming (32) has also proposed that the oxygen tension of the blood influences placental growth, based on observations of increased placental:fetal weight ratios in women with anemia caused by β-thalassemia minor. The hypothesis that high hemoglobin concentration in early pregnancy leads to poor placental development and increased risk of preterm birth should be pursued in larger studies.

The oxygen carrying capacity of the blood does not seem an adequate explanation for the relation between low hemoglobin and preterm birth. Early placental hyperplasia in anemic women (32) might increase the probability of an abrupted placenta in later pregnancy. However, other factors that influence birth weight through decreased oxygen supply to the fetus (e.g., smoking and high altitude) are more strongly associated with intrauterine growth retardation than with preterm birth (11). The observation by Scholl et al. (6) that only iron deficiency anemia, not other anemias, was related to preterm birth suggests that some iron-specific mechanism may be at play. The basis for this has not been studied (33).

The exclusion of a number of confounding factors in this study reduces the plausibility that anemia is acting as a marker for other maternal sociodemographic characteristics. However, it is possible that anemia is a marker for some other physiologic process that causes preterm birth. Uterine bleeding is associated with preterm birth and might cause anemia, but at week 7 of pregnancy, when hemoglobins were first measured in this study, the embryo has implanted for only 4 weeks. The opportunity seems small for bleeding in those 4 weeks to contribute substantially to the variation in hemoglobin in this sample. The later that hemoglobin is assessed in pregnancy, the greater the opportunity for uterine bleeding in pregnancy to cause anemia. In the study by Scholl et al. (6), anemia and vaginal bleeding were assessed in pregnancy week 17, but no association between these variables was found (RR of anemia associated with bleeding = 1.18, 95 percent CI 0.88–1.57). Unfortunately, uterine bleeding was not ascertained in this study, and therefore we cannot test the reverse hypothesis, i.e., that women who enter pregnancy with low hemoglobin are more prone to experience uterine bleeding. The basis for the relation between pregnancy anemia and preterm birth remains obscure.

Based on their observational study of pregnancy anemia and low birth weight, Steer et al. (10, p. 491) have recently stated that "hemoglobin concentrations <95 g/liter seem to be remarkably harmless." The difference between their conclusion and ours likely stems from their use of lowest pregnancy hemoglobin in their analysis. The lowest pregnancy hemoglobin value is influenced greatly by the adequacy of maternal plasma volume expansion, especially in the British population, where iron deficiency anemia in women is much less common than in Shanghai. As Steer et al. (10) point out, lowest pregnancy hemoglobin values of 100–110 g/liter likely reflect good maternal response to pregnancy more than poor iron status. Similarly, when lowest pregnancy hemoglobin value was examined in this population, the relative risk of low birth weight was close to unity at values ranging from 90–120 g/liter. Our results demonstrate that whether moderately low hemoglobin concentrations are harmless depends on when they occur in pregnancy.

Although several influential studies of this issue have been based on the lowest pregnancy hemoglobin value, the concept is illogical from a number of perspectives. The lowest value only has a reliable meaning if the number and timing of the measurements are standardized. This has not occurred in epidemiologic studies and is unlikely to be the case in clinical practice, because physicians will tend to reevaluate at closer intervals women who are at greater risk of anemia. The concept also lacks biologic validity, as hemoglobin concentrations in the second month of pregnancy mean something quite different from values in the sixth month of pregnancy, when plasma volume expansion is at its peak. As much as possible, continuing research in this area should measure hemoglobin at standard times of gestation to specifically test biologically plausible hypotheses. Unless hemoglobin concentration is deliberately used as a marker of plasma volume expansion, the best test of most hypotheses will involve hemoglobin measurements around conception or very early in pregnancy, when hemoglobin concentration does not reflect the changing plasma and red cell volume dynamics of pregnancy. The fact that the relation of hemoglobin concentration to preterm birth was strongest using early pregnancy measurements provides evidence that the relation does not derive from blood volume dynamics, but instead tends to be obscured by them.

In conclusion, this study provides strong evidence

175

that maternal anemia in early pregnancy increases the risk of preterm low birth weight. Because around half of the world's women are anemic, this finding has implications for programs to prevent low birth weight and subsequent infant mortality in many settings. A practical question is whether antenatal iron supplementation, which is often started in the second half of pregnancy, will reduce the rates of preterm low birth weight, or whether anemia plays a unique role in placental development during early pregnancy and therefore must be prevented prior to pregnancy. Other than smoking and urogenital infections, currently established risk factors for preterm birth are largely not preventable (13). This finding represents an opportunity for reducing preterm low birth weight and infant mortality, because iron deficiency, the most common cause of anemia globally (30), is both preventable and treatable.

ACKNOWLEDGMENTS

This work was funded in part through cooperative agreement no. DAN-5116-1-00-8051-00 between The Johns Hopkins University and the Office of Health and Nutrition, US Agency for International Development.

The authors thank Dr. James Tielsch for his helpful comments on the manuscript.

REFERENCES

1. United Nations Administrative Committee on Coordination—Subcommittee on Nutrition (ACC/SCN). Second report on the World nutrition situation. Vol 1. Global and regional Results. Geneva: ACC/SCN, 1992.
2. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985;312:82–90.
3. Hemminki E, Starfield B. Routine administration of iron and vitamins during pregnancy: review of controlled clinical trials. Br J Obstet Gynaecol 1978;85:404–10.
4. Routine iron supplementation during pregnancy. Policy statement. US Preventive Services Task Force. JAMA 1993;270: 2846–54.
5. Lu ZM, Goldenberg RL, Cliver SP, et al. The relationship between maternal hematocrit and pregnancy outcome. Obstet Gynecol 1991;77:190–4.
6. Scholl TO, Hediger ML, Fischer RL, et al. Anemia vs. iron deficiency: increased risk of preterm delivery in a prospective study. Am J Clin Nutr 1992;55:985–8.
7. Lieberman E, Ryan KJ, Monson RR, et al. Association of maternal hematocrit with premature labor. Am J Obstet Gynecol 1988;159:107–14.
8. Murphy JF, O'Riordan J, Newcombe RG, et al. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet 1986;1:992–4.
9. Garn SM, Ridella SA, Petzold AS, et al. Maternal hematologic levels and pregnancy outcomes. Semin Perinatol 1981;5: 155–62.
10. Steer P, Alam MA, Wadsworth J, et al. Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. Br Med J 1995;310:489–91.
11. Institute of Medicine. Nutrition during pregnancy. Washington, DC: National Academy Press, 1990.
12. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ 1987;65:663–737.
13. Berkowitz GS, Papiernik E. Epidemiology of preterm birth. Epidemiol Rev 1993;15:414–43.
14. Klebanoff MA, Shiono PH, Berendes HW, et al. Facts and artifacts about anemia and preterm delivery. JAMA 1989;262: 511–15.
15. Whittaker PG, MacPhail S, Lind T. Serial hematologic changes and pregnancy outcome. Obstet Gynecol 1996;88: 33–9.
16. Duffus GM, McGillivray I, Dennis KJ. The relationship between baby weight and changes in maternal weight, total body water, plasma volume electrolytes and proteins, and urinary oestriol excretion. J Obstet Gynaecol Br Cmwlth 1971;78: 97–104.
17. Gibson HS. Plasma volume and glomerular filtration rate in pregnancy and their relation to differences in fetal growth. J Obstet Gynaecol Br Cmwlth 1973;80:1067–74.
18. Alexander GR, Himes JH, Kaufman RB, et al. A United States national reference for fetal growth. Obstet Gynecol 1996;87: 163–8.
19. Yip R, Li Z, Chong W-H. Race and birth weight: the Chinese example. Pediatrics 1991;87:688–93.
20. Armitage P, Berry G. Statistical methods in medical research. 3rd ed. Oxford: Blackwell Scientific Publications, 1994.
21. Kahn HA. An introduction to epidemiologic methods. New York: Oxford University Press, 1983.
22. CDC criteria for anemia in children and childbearing-aged women. MMWR 1989;38:400–4.
23. Wen SW, Kramer MS, Usher RH. Comparison of birth weight distributions between Chinese and Caucasian infants. Am J Epidemiol 1995;141:1177–87.
24. Wen SW, Kramer MS, Hanley JA. Mean, deviants, and spread of distribution. Epidemiology 1995;6:639–40.
25. Lin C-C, Emanuel I. A comparison of American and Chinese intrauterine growth standards. Are American babies really smaller? Am J Epidemiol 1972;95:418–30.
26. Singh GK, Yu SM. Birthweight differentials among Asian Americans. Am J Public Health 1994;84:1444–9.
27. Fung KP, Wong TW, Lau SP. Ethnic determinants of perinatal statistics of Chinese: demography of China, Hong Kong and Singapore. Int J Epidemiol 1989;18:127–31.
28. Xi-He Z. Nutritional situation of Beijing residents. Southeast Asian J Trop Med Public Health 1992;23(Suppl 3):65–8.
29. Long-Qi X, Ze-Xiao J, Jia-Lun Y, et al. Soil-transmitted helminthiases: nationwide survey in China. Bull World Health Organ 1995;73:507–13.
30. Yip R. Iron deficiency: contemporary scientific issues and international programmatic approaches. J Nutr 1994;124: 1479S–1490S.
31. Wheeler T, Sollero C, Alderman S, et al. Relation between maternal haemoglobin and placental hormone concentrations in early pregnancy. Lancet 1994;343:511–13.
32. Fleming AF. Maternal anemia and fetal outcome in pregnancies complicated by thalassemia minor and "stomatocytosis." Am J Obstet Gynecol 1973;116:309–19.
33. Allen LH. Pregnancy and iron deficiency: unresolved issues. Nutr Rev 1997;55:91–101.

176

ZINC DEFICIENCY

Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival[1–3]

Laura E Caulfield, Nelly Zavaleta, Anuraj H Shankar, and Mario Merialdi

ABSTRACT Mild-to-moderate zinc deficiency may be relatively common worldwide, but the public health importance of this degree of zinc deficiency is not well defined. The purpose of this review was to provide a conceptual framework for evaluating the public health importance of maternal zinc deficiency as it relates to fetal growth and development, complications of pregnancy, labor and delivery, and maternal and infant health. The mechanisms through which zinc deficiency could influence health outcomes are well described. The results of experimental studies conducted in animal models have motivated concern about the potential health effects of mild-to-moderate maternal zinc deficiency. Observational studies in human populations have produced strong associations between poor maternal zinc status and various indicators of poor pregnancy outcome, but supplementation trials have not produced strong, or even consistent results. Supplementation trials are needed to define the public health importance of maternal zinc deficiency worldwide. *Am J Clin Nutr* 1998;68(suppl):499S–508S.

KEY WORDS Maternal zinc deficiency, pregnancy, child health, survival, supplementation, parturition, immunology, development

INTRODUCTION

In the past 20 y, much progress has been made in the area of child survival worldwide (1). Improvements in health care, water and sanitation, as well as socioeconomic development have substantially reduced the risks of childhood mortality. In many countries, a child's risk of dying during the first year of life remains high, but because efforts to prevent causes of infant death during the postneonatal period are becoming more and more successful, an ever-growing percentage of infant deaths occurs near the moment of birth. Therefore, efforts to further reduce mortality rates in developing countries will require public health planners and policymakers to place greater focus on understanding and addressing the causes of neonatal and perinatal mortality. Maternal nutritional status, with respect to both macro- and micronutrients, is known to be an important determinant of perinatal and neonatal survival and well-being (2), but maternal nutrition programs in developing countries have focused almost exclusively on the risks to the mother and infant from maternal iron deficiency anemia during pregnancy. Other micronutrients such as zinc may also be important for maternal and infant survival during the critical period surrounding birth, but their importance is less well understood. The purpose of this paper is to provide a background and conceptual framework for evaluating the public health importance of maternal zinc deficiency as it relates to fetal growth and development; complications of pregnancy, labor and delivery; and maternal and infant health and well-being.

PREVALENCE OF MATERNAL ZINC DEFICIENCY

Although severe zinc deficiency is now considered rare, mild-to-moderate zinc deficiency may be relatively common throughout the world (3). In general, few data are available on the prevalence of mild to moderate zinc deficiency worldwide, in part because consensus has not yet been reached on appropriate indicators of zinc status for pregnant women or any other target group (4, 5).

Several reports have compiled estimates of dietary zinc intakes of women worldwide. Tamura and Goldenberg (6) compiled 27 studies reporting dietary zinc intakes of women of childbearing age worldwide. Of these, 2 studies from developing countries [Guatemala and Egypt (7, 8)] reported average usual intakes of 8–11 mg Zn/d and 2 studies of intakes of south Asians living in the United Kingdom (9, 10) reported usual intakes of 5–10 mg Zn/d. Most of the studies cited were conducted in the United States and United Kingdom, and average usual zinc intakes were reported to be in the range 8–14 mg/d across the studies. In addition to these studies, Lehti (11) reported intakes of 7.0 mg Zn/d among lactating Brazilian women, and Ferguson et al (12) reported intakes of 6.2–6.8 mg Zn/d among Malawian women. Parr et al (13) summarized data from 17 surveys in the literature between 1970 and 1991 and reported that pregnant and lactating

[1] From the Center for Human Nutrition, the Department of International Health, and the Department of Molecular Microbiology and Immunology, The Johns Hopkins School of Public Health, Baltimore; and the Instituto de Investigación Nutricional (IIN), Lima, Perú.

[2] Supported by cooperative agreement no. DAN-5116-A-00-8051-00 and HRN-A-00-97-00015-00 between the US Agency for International Development Office of Health and Nutrition and The Johns Hopkins University.

[3] Address reprint requests to LE Caulfield, Center for Human Nutrition, Department of International Health, The Johns Hopkins School of Hygiene and Public Health, 615 North Wolfe Street, Room 2041, Baltimore, MD 21205. E-mail: lcaulfie@jhsph.edu.

The American Society for Clinical Nutrition Inc. does not endorse any commercial enterprise.

178

women worldwide consume 9.6 ± 1.2 mg Zn/d ($\bar{x}$ ± SD). Zinc may also be ingested from drinking water, but intakes are typically increased by only 2% provided there was a usual water intake of 2 L/d (13).

The probability method can be applied to these data to derive an estimate of the prevalence of inadequate intakes of zinc (14). Using the estimated usual intakes of Parr et al (13) and an estimated distribution of the zinc required by women to meet their normative needs during pregnancy of 11.5 ± 1.75 mg/d [based on the US recommended dietary allowances (15)], it can be estimated that 82% of pregnant women worldwide likely have inadequate usual intakes of zinc. This estimate is gross for several reasons. First, the average requirement is overestimated to the degree to which the typical female body size on which it is based is greater than that of women in developing countries. Second, the requirement distribution is calculated with the assumption that the dietary zinc is highly bioavailable. This results in an underestimation of the zinc intake requirement distribution for women in developing countries whose diets consist mainly of cereal grains and legumes and is therefore of moderate-to-low bioavailability with respect to zinc (13). Third, most of the studies were from developed countries with higher usual intakes of zinc. Despite these considerations, this estimate indicates an alarming prevalence that should motivate concern that significant proportions of pregnant women worldwide have inadequate dietary zinc intakes and likely suffer health consequences attributable to zinc deficiency.

CONSEQUENCES OF MATERNAL ZINC DEFICIENCY

Severe maternal zinc deficiency has been associated with spontaneous abortion and congenital malformations (ie, anencephaly), whereas milder forms of zinc deficiency have been associated with low birth weight (LBW), intrauterine growth retardation, and preterm delivery (16). Importantly, milder forms of zinc deficiency have also been related to complications of labor and delivery, including prolonged or inefficient first-stage labor and protracted second-stage labor, premature rupture of membranes (PROM), and the need for assisted or operative delivery (16). These complications in turn impair maternal and perinatal health because they lead to increased risk of maternal lacerations, high blood loss, maternal infections, fetal distress, stillbirth, neonatal asphyxia (low Apgar scores), respiratory distress, and neonatal sepsis (16). These interrelations are shown in **Figure 1**. The evidence relating maternal zinc deficiency to fetal growth and development, birth outcomes, and maternal and perinatal health are considered in the following sections.

Fetal growth

Women are at increased risk of zinc deficiency during pregnancy [in part because of high fetal requirements for zinc (17)], and maternal zinc deficiency has been associated with poor fetal growth in both animal and human populations (17, 18). In humans, however, the research relating maternal zinc status and birth weight has not produced consistent results. Of 38 studies reporting associations between maternal plasma or serum zinc

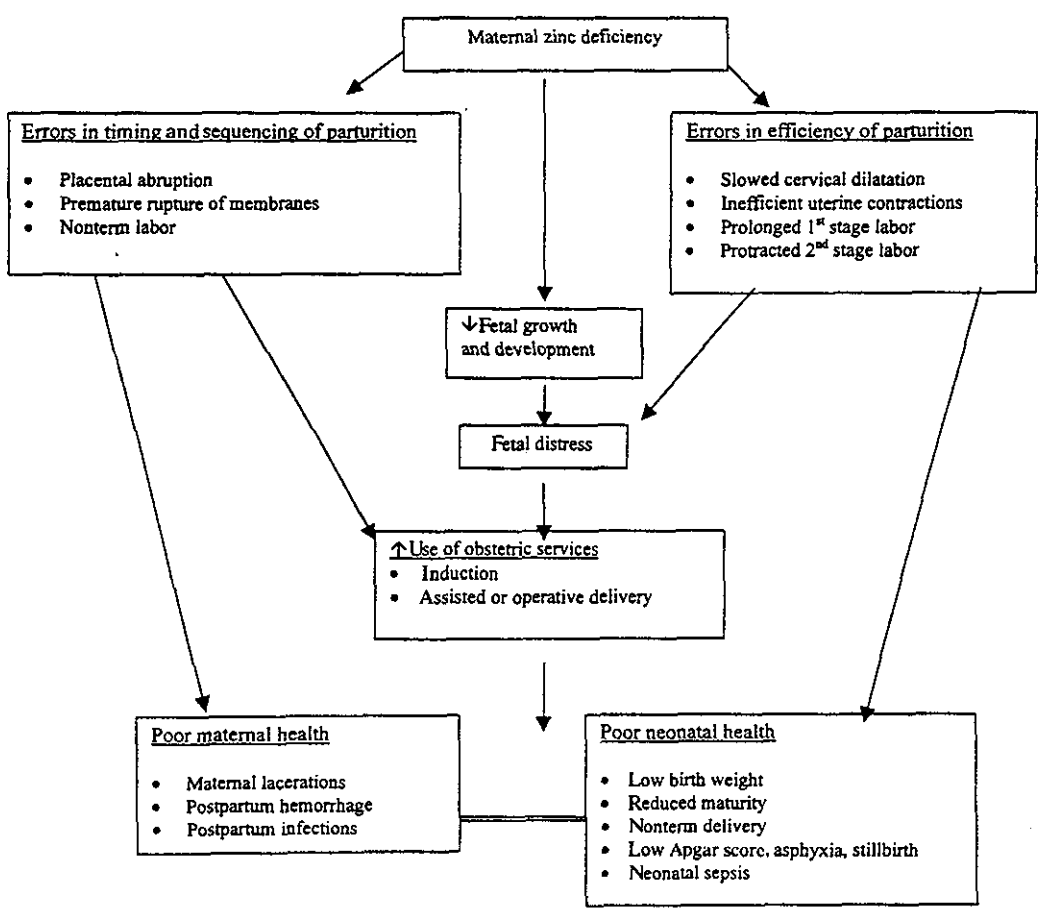


FIGURE 1. Consequences of maternal zinc deficiency on birth outcomes and maternal and perinatal health.

179

concentration and fetal growth (expressed either as birth weight or as some definition of adequacy, eg, birth weight >10th percentile), 18 studies (19–35) reported positive associations, whereas 20 studies (10, 36–54) reported negative associations. Of the 14 studies conducted in populations in developing countries, 9 reported positive associations (8, 20, 22, 26, 27, 28, 32–34) and 5 reported negative associations (37, 39, 40, 42, 48). Various reasons for these differences in associations can be postulated, including differences in *1*) timing of the assessment (blood draw) during pregnancy and the effects of hemodilution, *2*) laboratory methods and quality, *3*) sample size and the potential for influential values to drive associations, and *4*) the underlying zinc nutriture of the populations. It was also argued that the negative associations resulted because faster growing fetuses (with higher zinc requirements) drive down maternal serum concentrations (53).

The results of 10 zinc supplementation trials to improve birth weight in humans have also been mixed (55–65). The major problem with most of these trials is that they were conducted in well-nourished populations and thus are not addressing the central question of whether supplementing zinc-deficient women would improve fetal growth. Only 2 studies (57, 64) were conducted in poor women in developing countries. Ross et al (57) studied the pregnancy outcomes of 65 Zulu women in South Africa. Women receiving supplements containing between 4 and 13 mg Zn/d delivered babies weighing 80 g less at birth than those in the control group, but because mothers in the treatment group weighed considerably less than those in the control group at 20 wk gestation, the observed differences in birth weight may be explained. Garg et al (64) supplemented 106 Indian mothers with 45 mg Zn/d, and observed a 300–800 g difference in birth weight relative to a comparison group of 60 mothers. The differences between groups depended on the length of supplementation, with the greatest differences seen in those supplemented from the first trimester of pregnancy onward. Although these effects are remarkable, it is difficult to draw conclusions from this study because *1*) the sample size was small, *2*) the selection of the 60 mothers in the comparison group was not defined, *3*) there was a high loss to follow-up, and *4*) there was a lack of information on the comparability of the groups with respect to important confounding factors.

From an epidemiologic perspective, the trial with the greatest internal validity is that reported by Goldenberg et al (65), in which 580 low-income Alabaman women with low serum zinc concentrations at entry into prenatal care were randomly assigned to receive 25 mg Zn/d or placebo. Infants born to zinc-supplemented women weighed 126 g more at birth, were 0.6 cm longer, and had 0.4-cm larger head circumferences than infants born to mothers receiving the placebo. Further analyses revealed that the effects on fetal growth were limited to women who were not overweight when entering pregnancy. In this subgroup the results were much stronger, with a difference in birth weight between groups on the order of 250 g. How these findings can be generalized for women in developing countries is not clear, but the results should make clear the need for further research on this question to be conducted in developing country populations.

Despite the limitations of the trials discussed previously, except for the study by Ross et al (57), the birth weights of infants born to zinc-supplemented women in these trials were 14–800 g higher than those in the control groups, with 6 of the 10 trials reporting increases in mean birth weight of 40–170 g, and those conducted among high-risk subgroups of pregnant women showing the largest effects on birth weight. A 40–170 g increase in the average birth weight may seem small, but it is similar in magnitude to the effects on birth weight observed with energy and protein supplementation (66).

Several zinc supplementation trials reported differences in other indicators of fetal growth. Two trials reported 26–90% reductions in the incidence of small-for-gestational age infants (61, 64), and Goldenberg et al (65) reported a 37% reduction in the overall incidence of very LBW (<1500 g) associated with zinc supplementation and a 62% reduction in very LBW among nonobese women. Two studies reported that babies born to zinc supplemented mothers were 0.5 cm longer (62, 65), whereas one study (60) observed babies in the zinc supplemented group to be 0.2 cm shorter than control infants at birth. Zinc-supplemented women in Alabama had infants with 0.2 cm greater arm and femur lengths, and 1–2 mm greater triceps and subscapular skinfold thicknesses (6).

Length of gestation

Part of the reason for observed increases in birth weights in babies born to zinc-supplemented mothers could be the effects of zinc supplementation on the duration of pregnancy rather than on fetal growth per se. The duration of pregnancy in most animal species is not subject to much variation, but experimental maternal zinc deficiency has been shown in several species to increase variability in the duration of pregnancy—in essence, to create in those species the well-recognized human problems of preterm and postterm delivery (18, 67, 68). As will be discussed later, these effects likely reflect the role of zinc in estrogen-dependent gene expression as it relates to the timing, coordination, and progression of parturition (69).

The effects of zinc supplementation on duration of pregnancy and preterm delivery were examined in some but not all of the zinc-supplementation trials described above. Of 5 trials providing results (57, 59, 62, 64, 65), maternal zinc supplementation lengthened the average duration of pregnancy by 0.3–1.0 wk, with 3 of the trials reporting a lengthening of 0.5 wk. Three studies reported reductions in the incidence of preterm delivery (<37 wk) of 18–36% and 2 reported reductions of 80–83%. In 2 studies (16, 65) 66–85% reductions in the incidence of delivery before 32 wk were also reported. These results indicate a consistent, albeit small, effect of zinc supplementation on the average duration of pregnancy that likely explains most or all of the improvements in size at birth described previously. Thus, it may be true that increases in average birth weight observed with zinc supplementation occur not from improvements in fetal growth rates, but rather from prolongation of time spent in utero. However, it is also clear that maternal zinc supplementation likely results in more sizable reductions in preterm delivery, particularly in those deliveries occurring before 32 wk gestation. Finally, Kynast and Saling (62) reported an 80% reduction in the variance of gestational age at delivery, suggesting that the incidence of nonterm delivery (delivery at <37 wk or >41 wk) diminished with maternal zinc supplementation.

Neurobehavioral development

Zinc is a critical nutrient for central nervous system (CNS) development, which occurs during pre- and postnatal life, for example: *1*) zinc-dependent enzymes are involved in critical cell replication processes necessary for brain growth, *2*) zinc-finger

proteins provide brain structure and are important for neurotransmission, and *3*) zinc-dependent neurotransmitters in the mossy fiber system of the hippocampus are involved in brain memory function. In addition, zinc is involved in extra-CNS metabolic processes that ultimately affect CNS function, including hormone transport, receptor binding and metabolism, and neurotransmitter precursor production (70).

Because of the important role of zinc in CNS function, it is clear that maternal and early infant zinc deficiencies are likely to adversely affect fetal and infant neurologic and behavioral development (**Figure 2**). This is borne out in the work by Golub et al (70–74), who conducted a series of studies in rhesus monkeys investigating the effect of maternal and infant zinc deficiency on maternal and infant health outcomes, including fetal activity patterns, newborn motor development, and behavior patterns during infancy and pubescence. In these studies, the monkeys were exposed continually to marginally low concentrations of zinc (from the fetal period to adolescence) because this level of exposure would most greatly mimic zinc-deficiency in human populations. Using ultrasonographic techniques to quantify fetal movements, Golub et al (74) found that fetuses of zinc-deficient mothers had increased activity relative to their zinc-sufficient counterparts. However, newborns of zinc-deficient mothers were found to exhibit low postural muscle tone (hypotonia) but normal reflexes at birth (71). At 1 mo of age, the male infants were found to be less active, and lower activity levels associated with zinc deficiency were repeatedly observed in both sexes throughout childhood and into adolescence, despite a lack of differences in motor maturation between groups (72, 73). Finally, when cognitive performance tests were administered to the infants, the zinc-deficient infants exhibited reduced exploration and impaired visual discrimination learning performance, but improved spatial-delayed response performance—an expected finding in infants with lower activity levels. Taken together, the results of these studies overall suggest that maternal and infant zinc status influence in some way fetal and infant cognitive and behavioral development and highlight the need for studies in zinc-deficient human populations.

Case reports (75, 76) suggest that severe maternal zinc deficiency (associated with dietary deprivation or acrodermatitis enteropathica) increases the risk of neural tube defects in the offspring. As reported, maternal zinc supplementation allowed these women to produce healthy offspring in subsequent pregnancies. No experimental studies in humans have been conducted to evaluate the effect of mild-to-moderate maternal zinc deficiency on fetal and infant behavioral development. Two studies do provide evidence, however, of an important link between maternal zinc status and neonatal and infant behavior in humans. Kirksey et al (77) observed positive associations between maternal serum zinc concentrations during pregnancy and the developmental status of Egyptian newborns assessed by using the Brazelton Neonatal Behavioral Assessment Scale (78). Subsequently, Kirksey et al (8) reported that positive associations were still observed between maternal zinc status during pregnancy and infant developmental status when assessed at 6 mo of age by using the Bayley Scales of Infant Development (79).

Both the Brazelton (78) and the Bayley (79) tests are global assessments of developmental status, and therefore assess a variety of components of behavioral development and cognitive functioning. Importantly, the orientation and habituation components of both these tests were associated most strongly with

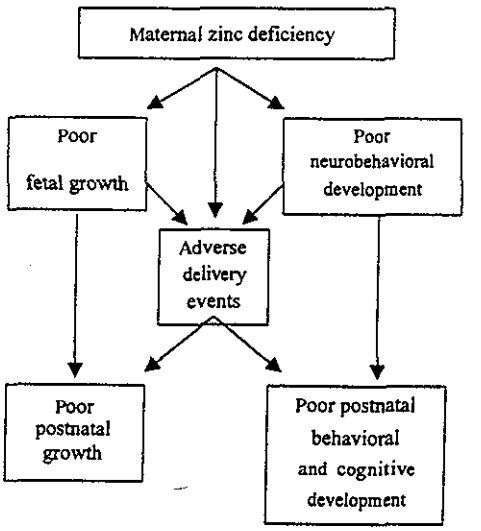


FIGURE 2. Interrelations between maternal zinc deficiency and pre- and postnatal growth and development.

maternal zinc status, and these are believed to relate directly to cognitive ability later in life (8). Orientation refers to the ability of the infant to respond appropriately to an environmental stimulus, whereas habituation refers to the ability of the infant to inhibit response to a repetitive or familiar environmental stimulus. More rapid habituation is generally interpreted as reflecting more focused attention and more efficient information processing by the infant. Speed of neonatal habituation has been related to measures of infant alertness, whereas measures of infant habituation have been shown to predict a variety of indexes of cognitive performance in the preschool years (80–82).

The associations were particularly strong between neonatal habituation measures and maternal zinc intakes and status during the second trimester (8). Given these results and knowledge that a phase of rapid neurogenesis and structural development occurs before the third trimester, it is reasonable to hypothesize that maternal zinc status during midpregnancy may be particularly important for ensuring optimal fetal CNS development and later cognitive functioning.

It should be added that dietary zinc insufficiency might not be necessary for adverse effects of zinc deficiency on CNS development to occur. The zinc pool in the mother's body that is transferred to the fetus is found in maternal plasma (83). Thus, factors known to influence maternal plasma zinc concentrations (other than chronically low dietary zinc intakes) may elicit the same CNS manifestations in the fetus seen in fetuses of mothers with low dietary intakes of zinc. Even transitory reductions in serum zinc may cause CNS malformations if they occur during organogenesis (84). Maternal plasma zinc concentrations are lowered as part of the acute-phase response of the body's immune system to disease, injury, or stress because zinc is sequestered away from the plasma and into maternal liver (85). Thus, it is hypothesized that frequent maternal morbidity or maternal stress may increase risk of poor neurologic development in the fetus by causing elicitation of acute-phase responses leading to sequestration of zinc from the plasma to the liver, where it is essentially unavailable for uptake by the fetus. Studies in animal models support the hypothesis that maternal injury and the associated acute-phase response result in preferential uptake of zinc by the maternal liver

[reviewed by Keen et al (85)]. If this is so, one would expect the benefits of maternal zinc supplementation on fetal and infant outcomes to differ depending on the morbidity burden experienced by the mother during pregnancy, and perhaps by the infant during postnatal life. To our knowledge, the importance of maternal zinc deficiency secondary to maternal morbidity has not been studied in human populations. Defining the importance of secondary zinc deficiency is likely critical for designing interventions in populations with high disease burdens in developing countries.

The work of Golub et al (70–74) also showed that differences in fetal neurologic development associated with maternal zinc deficiency persist to some degree postnatally. Other research in the developmental literature provides evidence for a relation between neurologic and motor development observed in utero with that observed during infancy, suggesting that developmental gains achieved during fetal life can be long lasting (86–88). Because research supports both the effect of variation in maternal nutritional status on fetal neurologic development as well as the postnatal permanence of neurologic development achieved in utero, it would be important to assess the effect of maternal zinc supplementation on fetal neurologic development and relate it to developmental status during infancy.

To summarize, despite both a clear biological role for zinc in CNS development and function and demonstration of behavioral effects from zinc deficiency in animal models, an assessment of the effects of mild-to-moderate maternal zinc deficiency on fetal and infant neurologic, behavioral, and cognitive development has never been undertaken.

Labor and delivery complications

Maternal mild-to-moderate zinc deficiency has been associated with increased risk of a variety of maternal and fetal complications of labor and delivery (26, 53, 54, 58–60). These can be categorized into *1*) abnormalities in labor and delivery, *2*) maternal morbidity during pregnancy and the puerperium, and *3*) perinatal morbidity. These associations likely reflect the role of zinc in maintaining immunocompetence, cell membrane integrity, prostaglandin synthesis and function, and estrogen-dependent gene expression.

The relation between maternal zinc nutriture and parturition was first described by Apgar (89). Female rats fed 1-μg Zn/g diet throughout pregnancy experienced difficult and prolonged labors as compared with control and pair-fed animals. Some zinc-deficient dams labored for 6 h or more and were exhausted or dead before delivering the entire litter. Hemorrhage, presumably due to uterine atony, placental abruption, or both, was also observed. The exhausted zinc-deficient females were less likely to eat the afterbirth and to clean up or care for their pups. Subsequently, Apgar (90) showed that zinc supplementation late in pregnancy could prevent difficulties in parturition in otherwise zinc-deficient dams. Since then, dystocia has been observed in zinc-deficient rats by other investigators as well as in zinc-deficient rhesus monkeys (91, 92). As described by Bunce et al (69), studies suggest that zinc deficiency results in normal estrogen concentrations late in gestation but decreased uterine estrogen receptors, uterine proteins, and the number of uterine gap junctions at delivery. These findings suggest that zinc deficiency impairs estrogen-dependent gene expression, not by interfering with estrogen production but rather by interfering with estrogen function via the estrogen receptor, which contains a zinc-finger protein. Thus, zinc is critical for the production of uterine proteins that convert the uterus from its passive state to one capable of concerted contractions with sufficient force to expel a fetus. Zinc nutriture may influence other aspects of labor and delivery as well (69), including hormonal control of cervical ripening and dilatation (93) and the maintenance of fetal membrane and amniotic fluid integrity (93–95).

If what is observed in animal models is relevant to human pregnancy, we would expect maternal zinc deficiency to upset both the sequencing and efficiency of parturition (as shown in Figure 1). Sequencing refers to the optimal ordering of events during parturition at term, including cervical dilatation, labor, rupture of membranes, and expulsion of the fetus and placenta. Efficiency refers to the duration of each stage of labor and delivery and the strength of uterine contractions. Thus, errors in sequencing include nonterm delivery (as discussed previously), PROM, and placental abruption, whereas errors in efficiency include prolonged first-stage labor (both latent and active), protracted second-stage labor, and prolonged third-stage labor.

Observational studies in humans have associated low maternal serum zinc concentrations during pregnancy or at delivery with PROM, placental abruption, inefficient uterine contractions, and prolonged or nonprogressive labor, all of which can result in the need for assisted (eg, forceps) or cesarean delivery. Maternal zinc deficiency has been associated with a 3.5–7.0-fold increased risk for PROM (96, 97), a 2.8-fold increased risk of placental abruption (62), a 2–5-fold increased risk for a prolonged first-stage labor (both latent and active phases), a 9-fold increased risk for a protracted second-stage labor, and a 4-fold increased risk for having a labor lasting >20 h (98, 99). Lazebnik et al (98) also found nearly a 5-fold increased risk of third-degree lacerations associated with poor maternal zinc status.

Supplementation trials (16, 60–63), however, have not found such strong effects. Only the results of Simmer et al (61) suggest that supplementing pregnant women with zinc can lower the risk of such complications of labor and delivery; they found 60–80% reductions in the incidences of induction and cesarean delivery associated with zinc supplementation. Again, these studies had design flaws and were not conducted in zinc-deficient populations.

The question as to whether maternal zinc deficiency increases the likelihood of labor and delivery complications is critical for developing countries in which women have little access to essential obstetric services. Prolonged labor as well as other complications of labor and delivery increase risk to both the mother and baby (100). Fetal survival at birth is a function of the labor and delivery process as well as the ability of the fetus to survive that process (100, 101). Although not clear-cut, some preterm infants may have increased chances of survival if born by cesarean delivery (102). Babies with LBW or intrauterine growth retardation are less likely to survive a prolonged birth process, and if they do survive, are more likely to suffer morbidity directly resulting from that prolonged birth process, eg, asphyxia (low Apgar score) and respiratory distress from meconium aspiration, asphyxiation, or both (100, 103). Sepsis, either maternal or neonatal, is a likely consequence of PROM, and maternal lacerations and hemorrhage increase a woman's risk of dying from blood loss or infection.

Few studies have examined maternal and perinatal morbidity associated with zinc deficiency. Mukherjee et al (54) found that, overall, women with low serum zinc concentrations were 20–50% more likely to suffer from a variety of maternal and fetal

complications of labor and delivery, including fetal distress and maternal infections. McMichael et al (53) reported a 1–2-fold increased risk of perinatal morbidities (low Apgar score, respiratory distress, fetal distress, or presence of meconium) associated with low maternal zinc status. In their quasi-experimental study, Kynast and Saling (62) found that zinc supplementation was associated with a 60% reduction in vaginal bleeding, placental abruption, and maternal hemorrhage. However, Mahomed et al (60), in a small supplementation trial, found no such effects.

In summary, problems with the current literature preclude drawing definitive conclusions regarding the importance of zinc supplementation during pregnancy as a public health intervention for preventing labor and delivery complications, and thereby ensuring maternal and neonatal health and well-being during the puerperium. The estimated effects from observational studies appear to be strong, but in many cases these effects are not significant because of small sample sizes. The results from supplementation trials are inconclusive because of major design flaws, which include sample sizes inadequate to detect differences in rare events. The implications of zinc deficiency for complications of labor and delivery are important for poor women living in developing countries, who are more likely to suffer negative consequences resulting from PROM, prolonged labor, maternal lacerations, and postpartum hemorrhage. These women are also more likely to have small or preterm babies who are less tolerant of a prolonged birth process, and both the mother and her baby will be less likely to either be treated for or recuperate from life-threatening maternal and perinatal morbidity.

Postnatal outcomes

Maternal zinc deficiency during pregnancy may result in problems for the infant during postnatal life. Effects from maternal zinc deficiency on growth and development in utero likely influence growth and development during neonatal life and beyond, as shown in Figure 2. Zinc is also important for the transfer of many substances to target tissues. Among these substances are 2 with recognized importance for child survival: immunologic factors and vitamin A. The interrelations between maternal zinc deficiency, infant vitamin A status, immunologic development, and postnatal health and survival are shown in **Figure 3**. These interrelations are dealt with in greater detail in other articles in this supplement (104, 105) and are discussed briefly below.

Immunologic development

A major determinant of postnatal survival is the ability of the infant to resist infection and respiratory and diarrheal disease. Because protective immunity develops as a result of exposure to pathogens, the newborn is at particularly high risk for infection during the first 6 mo of life when adaptive immunity is still not fully functional but exposure to invading pathogens is significant. Two factors, however, provide the infant with protection against pathogens. First, healthy infants develop natural immunity consisting of antibodies and T cells produced before infection that recognize common pathogens for respiratory and diarrheal disease. Second, the newborn has already received maternal antibodies in utero. These antibodies are not replenished after birth and their presence gradually decays to low values by 6 mo of age. In addition to providing protection, natural and maternal antibodies are believed to influence development of the emerging immune system in the child (106).

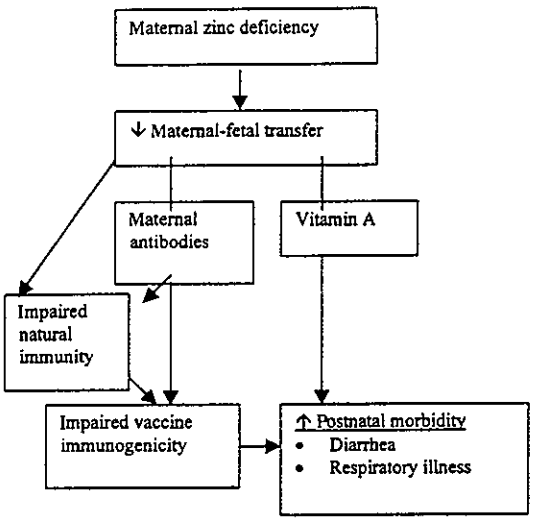


FIGURE 3. Potential links between maternal zinc deficiency and infant postnatal morbidity risk.

As discussed by Shankar and Prasad (104), perinatal zinc deficiency can result in poor development of natural immunity and decreased acquisition of maternal antibodies. Indeed, studies in mice and primates indicate that perinatal zinc deficiency can adversely affect the normal development of multiple tissues and organ systems of the fetus. One of the first sequelae of mild zinc deficiency is suppression of the immune response (107), indicating that the fetal immune system is particularly sensitive to suboptimal maternal zinc status. Perinatal zinc deficiency has been shown to result in decreased spleen and thymus size (108), impaired lymphocyte mitogenic responses and plaque forming activity (108), and depressed immunoglobulin concentrations, particularly for immunoglobulin M and immunoglobulin A (108, 109). Interestingly, the changes in immunoglobulin M and immunoglobulin A concentrations and some B cell responses persist into adulthood even after postnatal restoration of normal dietary zinc intake. In some cases they are passed on to subsequent generations (108–110). Because zinc is a necessary cofactor for the transport of immunoglobulins across the placental barrier, perinatal zinc deficiency may also diminish in utero acquisition of maternal antibodies by the fetus.

Given the central role of immunity in health and development, perinatal zinc deficiency may be a previously unrecognized cause of impaired disease resistance and decreased vaccine efficacy in infants. The ultimate effect of these sequelae on incidence, severity, and duration of diarrheal and respiratory disease is not known. Hence, research is needed on the influence of perinatal zinc deficiency on immunity and subsequent risk of morbidity as well as on the efficacy of prenatal maternal zinc supplementation and postnatal zinc-based therapies to boost protective immunity in neonates.

Vitamin A status

The relation between zinc and vitamin A status is well known (111). As reviewed by Christian and West (105), previous studies provide some indication that maternal zinc deficiency may influence newborn vitamin A status. Mild gestational zinc deficiency in pregnant rhesus monkeys was associated with lower maternal serum retinol and a lack of association between serum

183

retinol and retinol binding protein (112). In a related study, Peters et al (113) found reduced vitamin A concentrations in fetuses of mothers fed a diet throughout pregnancy that was marginal in zinc, but replete in vitamin A. Furthermore, vitamin A supplementation of these mothers did not overcome the deleterious effects of zinc deficiency on maternal transfer of vitamin A to the fetus. Given the well-documented, critical role of vitamin A for child survival (114), studies are needed to determine the public health importance of maternal zinc deficiency on vitamin A metabolism during pregnancy and lactation, and in particular, on delivery of vitamin A to the fetus and young infant.

Postnatal growth

The effect of zinc deficiency on growth has been well studied. Both animal and human studies provide evidence for the crucial role of zinc in supporting adequate growth, and results from supplementation trials in preschool and school-aged children support the public health importance of zinc deficiency in growth faltering during infancy and childhood (115). Research also suggests that the proportion of growth faltering attributable to zinc deficiency is likely greater in regions characterized by poor water and sanitation and hygiene practices that increase risk for diarrheal and respiratory infections, both of which increase the likelihood of zinc deficiency by increasing body losses or requirements (116).

The work of Golub et al (71) in rhesus monkeys suggests that growth faltering associated with maternal zinc deficiency during fetal life lasts throughout infancy. To our knowledge, no studies in human populations have been reported to determine whether improvements in birth weight and other anthropometric indexes associated with maternal zinc supplementation are long lasting. It may be true that improvements in birth weight achieved through maternal zinc supplementation in developing country settings are short-lived because the infants are born into environments characterized by high morbidity and diets of marginal quality and lose some or all of the gains achieved in utero. Alternatively, it is plausible that improvements in prenatal growth translate into even greater improvements in postnatal growth, with improvements in immune function and decreased risk of morbidity playing key roles in this process. Documenting the extent and duration of postnatal benefits to the infant from maternal zinc supplementation during pregnancy would be important for strengthening support for maternal nutrition programs and for understanding potential tradeoffs with infant-based supplementation programs when resources are scarce.

SUMMARY

Nutritionists have long been concerned that zinc deficiency affects large numbers of women and children worldwide. Little direct evidence of mild-to-moderate zinc deficiency is available, however, because the validity of biochemical indicators of zinc status are not established. There is considerable evidence that a high percentage of women worldwide consume diets inadequate in zinc; we estimated, albeit grossly, that 82% of pregnant women worldwide have usual intakes of zinc inadequate to meet the normative needs of pregnancy. In developing countries, the prevalence is likely to be near 100%. Therefore, program planners and policymakers should be concerned about maternal zinc deficiency in their populations, and the likely health burden for women and infants attributable to mild-to-moderate maternal zinc deficiency.

The purpose of this review was to define the range of potential health consequences for women and infants attributable to maternal zinc deficiency. As discussed above, the molecular roles of zinc in various biochemical processes relevant to maternal and infant health and survival are well established. Further, the results of experimental studies conducted in animal models including nonhuman primates suggest that maternal zinc deficiency does undermine several biological processes that ensure maternal and fetal well-being during pregnancy, parturition, and the puerperium, as well as infant health and survival in the first year of life and beyond. Observational studies conducted in human populations have found strong associations between various indicators of maternal zinc status and important health outcomes, including fetal growth and development, birth outcomes, and maternal and perinatal morbidity. However, outcomes such as infant immunologic development have not yet been investigated in human populations. For the most part, the results of the observational studies are provocative, but do not provide conclusive evidence about the role of maternal zinc deficiency in maternal and infant health because of methodologic flaws inherent in these studies. Further, because we are not sure what indicators of maternal zinc status mean, supplementation trials should be relied on more heavily to define maternal zinc deficiency, as well as the functional consequences of maternal zinc deficiency for women and infants. Unfortunately, the results of supplementation trials currently in the literature do not allow us to draw definitive conclusions.

It should be clear from the material presented that maternal zinc deficiency may be a triple threat to poor women in developing countries. These women are most likely to *1*) be carrying a small fetus unable to withstand prolonged labor and delivery, *2*) have the least access to assisted or operative delivery as treatment for prolonged labor and delivery, and *3*) have the least access to health care for proper treatment of maternal and perinatal morbidity during the puerperium. However, the contribution of maternal zinc deficiency to maternal and infant morbidity and mortality rates in developing countries is not known. Supplementation trials are needed to define the health consequences for mothers and infants attributable to maternal zinc deficiency worldwide as well as the potential benefits to maternal and infant well-being and survival likely to result from maternal zinc supplementation programs. Currently, there are trials ongoing in several countries in Latin America and Asia to define the health consequences of maternal zinc deficiency. Thus, it is likely that within the next 2–5 y, nutrition policymakers will begin to have the data necessary to make decisions regarding the public health importance of maternal zinc deficiency and potential programmatic options for reducing the morbidity and mortality burden attributable to maternal zinc deficiency worldwide.

REFERENCES

1. United Nations Children's Fund. The state of the world's children, 1995. New York: Oxford University Press, 1995.
2. Koblinsky MA. Beyond maternal mortality—magnitude, interrelationship, and consequences of women's health, pregnancy-related complications and nutritional status on pregnancy outcomes. Int J Gynaecol Obstet 1995;48:S21–32.
3. Sandstead HH. Zinc deficiency: a public health problem? Am J Dis Child 1991;145:853–9.
4. Solomons NW. Biological availability of zinc in humans. Am J Clin Nutr 1982;35:1048–75.

5. Aggett PJ. The assessment of zinc status: a personal view. Workshop on assessment of zinc status. Proc Nutr Soc 1991;50:9–17.
6. Tamura T, Goldenberg RL. Zinc nutriture and pregnancy outcome. Nutr Res 1996;16:139–81.
7. Fitzgerald SL, Gibson RS, Quan de Serrano J, et al. Trace element intakes and dietary phytate/Zn and Ca × phytate/Zn millimolar ratios of periurban Guatemalan women during the third trimester of pregnancy. Am J Clin Nutr 1993;57:195–201.
8. Kirksey A, Wachs TD, Yunis F, et al. Relation of maternal zinc nutriture to pregnancy outcome and infant development in an Egyptian village. Am J Clin Nutr 1994;60:782–92.
9. Abraham R, Campbell-Brown M, Haines AP, North WRS, Hainsworth V, McFadyen IR. Diet during pregnancy in an Asian community in Britain—energy, protein, zinc, copper, fibre and calcium. Hum Nutr Appl Nutr 1985;39:23–35.
10. Campbell-Brown M, Ward RJ, Haines AP, et al. Zinc and copper in Asian pregnancies—is there evidence for a nutritional deficiency? Br J Obstet Gynaecol 1985;92:875–85.
11. Lehti KK. Iron, folic acid and zinc intakes and status of low socioeconomic pregnant and lactating Amazonian women. Eur J Clin Nutr 1989;43:505–13.
12. Ferguson EL, Gadowshy SL, Huddle J-M, Cullinan TR, Lehrfeld J, Gibson RS. An interactive 24-h recall technique for assessing the adequacy of trace mineral intakes of rural Malawian women; its advantages and limitations. Eur J Clin Nutr 1995;49:565–78.
13. Parr RM. Assessment of dietary intakes. In: Trace elements in human nutrition and health. Geneva: World Health Organization, 1996:265–88.
14. National Research Council. The probability approach. In: Nutrient adequacy—assessment using food consumption surveys. Washington, DC: National Academy Press, 1986:25–40.
15. National Research Council. Recommended Dietary Allowances. 10th ed. Washington, DC: National Academy Press, 1989.
16. Jameson S. Zinc status in pregnancy: the effect of zinc therapy on perinatal mortality, prematurity, and placental ablation. Ann N Y Acad Sci 1993;678:178–92.
17. Swanson CA, King JC. Zinc and pregnancy outcome. Am J Clin Nutr 1987;46:763–71.
18. Apgar J. Zinc and reproduction. Annu Rev Nutr 1985;5:43–68.
19. Crosby WM, Metcoff J, Costiloe JP, et al. Fetal malnutrition: an appraisal of correlated factors. Am J Obstet Gynecol. 1977;128:22–9.
20. Atinmo T, Mbofung C, Osinusi BO. Relationship of zinc and copper concentrations in maternal and cord blood and birth weight. Int J Gynaecol Obstet 1980;18:452–4.
21. Ghosh A, Fong LYY, Wan CW, Liang ST, Woo JSK, Wong V. Zinc deficiency is not a cause for abortion, congenital abnormality and small-for-gestational age infants in Chinese women. Br J Obstet Gynaecol 1985;92:886–91.
22. Ette A, Ibeziako PA. Plasma zinc and copper concentrations in pregnant Nigerian women and newborns. Afr J Med Med Sci 1985;14:99–103.
23. Simmer K, Thompson RPH. Zinc in the fetus and newborn. Acta Paediatr Scand Suppl 1985;319:158–63.
24. Neggers YH, Cutter GR, Acton RT, et al. A positive association between maternal serum zinc concentration and birth weight. Am J Clin Nutr 1990;51:678–84.
25. Neggers YH, Cutter GR, Alvarez JO, Goldenberg RL. The relationship between maternal serum zinc levels during pregnancy and birthweight. Early Hum Dev 1991;25:75–85.
26. Mameesh MS, Hathout H, Safar MAAI, Mahfouz A, Al-Hassan JM. Maternal plasma proteins. Magnesium, zinc and copper concentration at term associated with birth size in Kuwait. Acta Vitaminol Enzymol 1985;7:183–8.
27. Singh PP, Khushlani K, Veerwal PC, Gupta RC. Maternal hypozincemia and low-birth weight infants. Clin Chem 1987;33:1950 (letter).
28. Kapoor RK, Misra PK, Dixit S, Wakhlu I, Sharma B, Seth TD. Zinc and intrauterine growth. Indian Pediatr 1988;25:972–6.
29. Meadows NJ, Ruse W, Smith MF, et al. Zinc and small babies. Lancet 1981;2:1135–7.
30. Higashi A, Tajiri A, Matsukura M, Matsuda I. A prospective survey of serial maternal serum zinc levels and pregnancy outcome. J Pediatr Gastroenterol Nutr 1988;7:430–3.
31. Okonofua FE, Amole FA, Emofurieta WO, Ugwu NC. Zinc and copper concentration in plasma of pregnant women in Nigeria. Int J Gynaecol Obstet 1989;29:19–23.
32. Yasodhara P, Ramaraju LA, Raman L. Trace minerals in pregnancy. 1. Copper and zinc. Nutr Res 1991;11:15–21.
33. Jeswani RM, Vani SN. A study of serum zinc levels in cord blood of neonates and their mothers. Indian J Pediatr 1991;58:683–7.
34. Bahl L, Chaudhuri LS, Pathak RM. Study of serum zinc in neonates and their mothers in Shiml hills (Himachal Pradesh). Indian J Pediatr 1994;61:571–5.
35. Ortega RM, Andres P, Martinez RM, Lopez-Sobaler AM, Quintas ME. Zinc levels in maternal milk: the influence of nutritional status with respect to zinc during the third trimester of pregnancy. Eur J Clin Nutr 1997;51:253–8.
36. Bogden JD, Thind IS, Kemp FW, Caternin H. Plasma concentrations of calcium, chromium, copper iron, magnesium, and zinc in maternal and cord blood and their relationship to low birth weight. J Lab Clin Med 1978;92:455–62.
37. Prema K. Predictive value of serum copper and zinc in normal and abnormal pregnancy. Indian J Med Res 1980;71:554–60.
38. Tuttle S, Aggett PJ, Campbell D, MacGillivray I. Zinc and copper nutrition in human pregnancy: a longitudinal study in normal primagravidae and in primagravidae at risk of delivering a growth retarded baby. Am J Clin Nutr 1985;41:1032–41.
39. Fehily D, Fitzsimmons B, Jenkins D, Cremin FM, Flynn A, Soltan MH. Association of fetal growth with elevated maternal plasma zinc concentration in human pregnancy. Hum Nutr Clin Nutr 1986; 40C:221–7.
40. Adeniyi FAA. The implications of hypozincemia in pregnancy. Acta Obstet Gynecol Scand 1987;66:579–82.
41. Jepsen LV, Clemmensen K. Zinc in Danish women during late normal pregnancy and pregnancies with intra-uterine growth retardation. Acta Obstet Gynecol Scand 1987;66:401–5.
42. Okonofua FE, Isinkaye A, Onwudiegwu U, Amole FA, Emofurieta WA, Ugwu NC. Plasma zinc and copper in pregnant Nigerian women at term and their newborn babies. Int J Gynaecol Obstet 1990;32:243–5.
43. Bro S, Berendtsen H, Nørgaard J, Høst A, Jørgensen PJ. Serum zinc and copper concentrations in maternal and umbilical cord blood. Relation to course and outcome of pregnancy. Scand J Clin Lab Invest 1988;48:805–11.
44. Marsal K, Furgyik S. Zinc concentrations in maternal blood during pregnancy and postpartum, in cord blood and amniotic fluid. Acta Obstet Gynecol Scand 1987;66:653–6.
45. Lao TT, Loong EPL, Chin RKH, Lam CWK, Lam YM. Zinc and birth weight in uncomplicated pregnancies. Acta Obstet Gynecol Scand 1990;69:609–11.
46. Lao TT, Chin RKH, Mak YT, Swaminatan R, Lam YM. Plasma and erythrocyte zinc and birth weight in pre-eclamptic pregnancies. Arch Gynecol Obstet 1990;247:167–71.
47. Wasowicz W, Wolkanin P, Begnarski M, Gromadzinska J, Sklodowska M, Grzybowska K. Plasma trace element (Se, Zn, Cu) concentrations in maternal and umbilical cord blood in Poland. Biol Trace Elem Res 1993;38:205–15.
48. Islam MA, Hemalatha P, Bhaskaram P, Kumar PA. Leukocyte and plasma zinc in maternal and cord blood: their relationship to period of gestation and birth weight. Nutr Res 1994;14:353–60.
49. Tamura T, Goldenberg RL, Freeberg LE, Cliver SP, Cutter GR, Hoffman HJ. Maternal serum folate and zinc concentrations and their relationships to pregnancy outcome. Am J Clin Nutr 1992;56:365–70.
50. Wolfe SA, Gibson RS, Gadowsky SL, O'Connor DL. Zinc status of

185

a group of pregnant adolescents at 36 wks gestation living in southern Ontario. J Am Coll Nutr 1994;13:154–64.
51. Carbone P, Sobreviela M, Jimenez D, et al. Hair zinc and dietary zinc intake during pregnancy and puerperium. Eur J Obstet Gynecol Reprod Biol 1992;47:103–8.
52. Metcoff J, Costiloe J, Crosby W, et al. Maternal nutrition and fetal outcome. Am J Clin Nutr 1981;34:708–21.
53. McMichael AJ, Dreosti IE, Gibson GT, Hartshorne JM, Buckley RA, Colley DP. A prospective study of serial maternal serum zinc levels and pregnancy outcome. Early Hum Dev 1982;7:59–69.
54. Mukherjee MD, Sandstead HH, Ratnaparkhi MV, Johnson LK, Milne DB, Stelling HP. Maternal zinc, iron, folic acid, and protein nutriture and outcome of human pregnancy. Am J Clin Nutr 1984;40:496–507.
55. Jameson S. Zinc status and pregnancy outcomes in humans. In: Prasad AS, Dreosti IE, Hetzel BS, eds. Clinical applications of recent advances in zinc metabolism, New York: Alan R Liss, 1982:39–52.
56. Hunt IF, Murphy NJ, Cleaver AE, et al. Zinc supplementation during pregnancy: effects on selected blood constituents and on progress and outcome of pregnancy in low-income women of Mexican descent. Am J Clin Nutr 1984;40:508–21.
57. Ross SM, Nel E, Naeye R. Differing effects of low and high bulk maternal dietary supplements during pregnancy. Early Hum Dev 1985;10:295–302.
58. Hunt IF, Murphy NJ, Cleaver AE, et al. Zinc supplementation during pregnancy in low-income teenagers of Mexican descent: effects on selected blood constituents and on progress and outcome of pregnancy. Am J Clin Nutr 1985;42:815–28.
59. Cherry FF, Sandstead HH, Rojas P, Johnson LK, Batson HK, Wang XB. Adolescent pregnancy: associations among body weight, zinc nutriture, and pregnancy outcome. Am J Clin Nutr 1989;50:945–54.
60. Mahomed K, James DK, Golding J, McCabe R. Zinc supplementation during pregnancy: a double blind randomised controlled trial. Br Med J 1989;299:826–30.
61. Simmer K, Lort-Phillips L, James C, Thompson RPH. A double-blind trial of zinc supplementation in pregnancy. Eur J Clin Nutr 1991;45:139–44.
62. Kynast G, Saling E. Effect of oral zinc application during pregnancy. Gynecol Obstet Invest 1986;21:117–23.
63. Robertson JS, Heywood B, Atkinson SM. Zinc supplementation during pregnancy. J Public Health Med 1991;13:227–9.
64. Garg HK, Singhla KC, Arshad Z. A study of the effect of oral zinc supplementation during pregnancy on pregnancy outcome. Indian J Physiol Pharmacol 1993;37:276–84.
65. Goldenberg RL, Tamura T, Neggers Y, et al. The effect of zinc supplementation on pregnancy outcome. JAMA 1995;274:463–8.
66. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organization 1987;65:663–737.
67. Apgar J, Fitzgerald JA. Effect on the ewe and lamb of low zinc intake throughout pregnancy. J Anim Sci 1985;60:1530–8.
68. Apgar J, Everett GA. Low zinc intake affects maintenance of pregnancy in guinea pigs. J Nutr 1991;121:192–200.
69. Bunce GE, Lytton F, Gunesekera B, Vessal M, Kim C. Molecular basis for abnormal parturition in zinc deficiency in rats. In: Allen LH, King J, Lonnerdal B, eds. Nutrient regulation during pregnancy, lactation, and infant growth. New York: Plenum Press, 1994:209–14.
70. Golub M, Keen CL, Gershwin ME, Hendrickx AG. Developmental zinc deficiency and behavior. J Nutr 1995;125:2263S–71S.
71. Golub M, Gershwin ME, Hurley LS, Saito WY, Hendrickx AG. Studies on marginal zinc deprivation in rhesus monkeys: IV. Growth of infants in the first year of life. Am J Clin Nutr 1984;40:1192–202.
72. Golub MS, Gershwin ME, Hurley LS, Hendrickx AG, Saito WY. Studies of marginal zinc deprivation in rhesus monkeys: infant behavior. Am J Clin Nutr 1985;42:1229–39.
73. Golub MS, Gershwin ME, Hurley LS, Hendrickx AG. Studies of marginal zinc deprivation in rhesus monkeys. VIII. Effects on early adolescence. Am J Clin Nutr 1988;47:1046–51.
74. Golub MS, Tarantel AF, Gershwin ME, Keen CL, Hendrickx AG, Lonnerdal B. Ultrasound evaluation of fetuses of zinc-deprived monkeys (*Macaca mulatta*). Am J Clin Nutr 1992;55:734–40.
75. Cavdar AO, Bahceci M, Akar N, Erten J, Yahuz H. Effect of zinc supplementation in a Turkish woman with two previous anacephalic infants. Gynecol Obstet Invest 1991;47:103–8.
76. Hambidge KM, Nelder KH, Walravens PA. Zinc, acrodermatitis enteropathica, and congenital malformations. Lancet 1975;1:577–8.
77. Kirksey A, Rahmanifar A, Wachs TD, et al. Determinants of pregnancy outcome and newborn behavior of a semirural Egyptian population. Am J Clin Nutr 1991;54:657–67.
78. Brazelton TB. Neonatal behavior assessment scale. 2nd ed. London: Spastics International Medical Publications, 1984.
79. Bayley N. Bayley scales of infant development manual. New York: Psychological Corporation, 1969.
80. Isabella R, Ward M, Belsky J. Convergence of multiple sources of information on infant individuality. Infant Behav Dev 1985;8:283–91.
81. Bornstein MH, Sigman MD. Continuity in mental development from infancy. Child Dev 1986;57:251–74.
82. McCall RB, Carriger MS. A meta-analysis of infant habituation and recognition memory performance as predictors of later IQ. Child Dev 1993;64:57–79.
83. World Health Organization. Zinc. In: Trace elements in human nutrition and health. Geneva: WHO, 1996:72–104.
84. Wallwork C, Sandstead HH. Zinc and brain function. In: Prasad A, ed. Essential and toxic trace elements in human health and disease: an update. New York: Wiley-Liss, 1993:65–80.
85. Keen CL, Taubeneck MW, Daston GP, Rogers JM, Gershwin ME. Primary and secondary zinc deficiency as factors underlying abnormal CNS development. Ann N Y Acad Sci 1993;678:37–47.
86. Eaton WO, Saudino KJ. Prenatal activity level as a temperament dimension? Individual differences and developmental functions in fetal movement. Infant Behav Dev 1992;15:57–70.
87. Madison LS, Madison JK, Adubato SA. Infant behavior and development in relation to fetal movement and habituation. Child Dev 1986;57:475–82.
88. Shadmi A, Homburg R, Insler V. An examination of the relationship between fetal movements and infant motor activity. Acta Obstet Gynecol Scand 1986;65:335–9.
89. Apgar J. Effect of zinc deficiency on parturition in the rat. Am J Physiol 1968;215:160–3.
90. Apgar J. Effect of zinc repletion late in gestation on parturition in the zinc-deficient rat. J Nutr 1973;103:973–81.
91. Bunce GE, Wilson GR, Mills CF, Klopper A. Studies on the role of zinc in parturition in the rat. Biochem J 1983;210:761–7.
92. Golub MS, Gershwin ME, Hurley LS, Baly DL, Hendrickx AG. Studies of marginal zinc deprivation in rhesus monkeys: II. Pregnancy outcome. Am J Clin Nutr 1984;39:879–87.
93. Olson DM, Mijovic JE, Sadowsky DW. Control of human parturition. Semin Perinatol 1995;19:52–63.
94. Kynast G, Saling E. The relevance of zinc in pregnancy. J Perinat Med 1980;8:171–82.
95. Cunnane SC. Foetal mortality in moderately zinc-deficient rats is strictly related to the process of parturition: effect of maternal essential fatty acid supplementation. Br J Nutr 1982;47:495–504.
96. Sikorski S, Juszkiewicz T, Paszkowski T. Zinc status in women with premature rupture of membranes at term. Obstet Gynecol 1990;76:675–7.
97. Scholl TO, Hediger ML, Schall JI, Fischer RL, Khoo CS. Low zinc intake during pregnancy: its association with preterm and very preterm delivery. Am J Epidemiol 1993;137:1115–24.
98. Lazebnik N, Kuhnert BR, Kuhnert PM, Thompson KL. Zinc status, pregnancy complications, and labor abnormalities. Am J Obstet Gynecol 1988;158:161–6.

186

99. Dura-Trave T, Puig-Abuli M, Monreal I, Villa-Elizaga I. Relation between maternal plasmatic zinc levels and uterine contractility. Gynecol Obstet Invest 1984;17:247–51.
100. Chelmow D, Kilpatrick SJ, Laros RK. Maternal and neonatal outcomes after prolonged latent phase. Obstet Gynecol 1993;81:486–91.
101. Grant A, Elbourne D. Fetal movement counting to assess fetal well-being. In: Chalmers I, Enkin M, Keirse MJNC, eds. Effective care in pregnancy and childbirth. New York: Oxford Medical Publications, 1989:440–54.
102. Chao C. Factors affecting optimal mode of delivery. In: Witter F, Keith L, eds. Textbook of prematurity. Boston: Little Brown, 1993:203–22.
103. Caulfield LE, Haas JD, Belizán JM, Rasmussen KM, Edmonston B. Differences in early postnatal morbidity risk by pattern of fetal growth in Argentina. Paediatr Perinat Epidemiol 1991;5:263–75.
104. Shankar AH, Prasad AS. Zinc and immune functions: the biological basis of altered resistance to infection. Am J Clin Nutr 1998;68(suppl):447S–63S.
105. Christian P, West KP. Interactions of zinc and vitamin A: an update. Am J Clin Nutr 1998;68(suppl):435S–41S.
106. Herzenberg LA, Kantor AB, Herzenberg LA. Layered evolution in the immune system. A model for the ontogeny and development of multiple lymphocyte lineages. Ann N Y Acad Sci 1992;651:1–9.
107. Keen CL, Gershwin ME. Zinc deficiency and immune function. Annu Rev Nutr 1990;10:415–31.
108. Beach RS, Gershwin ME, Hurley LS. The reversibility of developmental retardation following murine fetal zinc deprivation. J Nutr 1982;112:1169–81.
109. Beach RS, Gershwin ME, Hurley LS. Gestational zinc deprivation in mice: persistence of immunodeficiency for three generations. Science 1982;218:469–71.
110. Beach RS, Gershwin ME, Hurley LS. Persistent immunological consequences of gestation zinc deprivation. Am J Clin Nutr 1983; 38:579–90.
111. Solomons NW, Russell RM. The interaction of vitamin A and zinc: implications for human nutrition. Am J Clin Nutr 1980;33:2031–40.
112. Baly DL, Golub MS, Gershwin ME, Hurley LS. Studies of marginal zinc deprivation in rhesus monkeys. III. Effects on vitamin A metabolism. Am J Clin Nutr 1984;40:199–207.
113. Peters AJ, Keen CL, Lonnerdal B, Hurley LS. Zinc-vitamin A interaction in pregnant and fetal rats: high vitamin A does not compensate. J Nutr 1986;116:1765–71.
114. Sommer A, West KP. Child survival. In: Vitamin A deficiency: health, survival, and vision. New York: Oxford University Press, 1996:19–61.
115. Allen LH. Nutritional influences on linear growth: a general review. Eur J Clin Nutr 1994;48(S1):S75–89.
116. Sazawal S, Black RE, Bhan MK, Jalla S, Sinha A, Bhandari N. Zinc supplementation in young children with acute diarrhea in India. N Engl J Med 1995;333:839–44.

Interactions between zinc and vitamin A: an update[1–4]

Parul Christian and Keith P West Jr

ABSTRACT Zinc status influences several aspects of vitamin A metabolism, including its absorption, transport, and utilization. Two common mechanisms postulated to explain this dependence relate to *1*) the regulatory role of zinc in vitamin A transport mediated through protein synthesis, and *2*) the oxidative conversion of retinol to retinal that requires the action of a zinc-dependent retinol dehydrogenase enzyme. However, evidence of an effect of zinc intake on vitamin A status from animal experiments is inconclusive, mainly because of the use of inadequate control groups. The higher weight gain of control animals as compared with the zinc-deficient ones in these experiments, even though pair fed, makes it difficult to isolate effects of zinc deficiency per se from those of generalized protein-energy malnutrition. A curvilinear relation has been suggested to describe an effect of plasma zinc on vitamin A transport. In humans, cross-sectional studies have more often than not shown a weak linkage between vitamin A and zinc status. Randomized trials have failed to show a consistent effect of zinc supplementation on vitamin A status. In disease states in which liver function is severely compromised and both zinc and vitamin A metabolism and transport are impaired, serum zinc and vitamin A concentrations tend to be positively correlated. In conclusion, clear evidence of synergy between these 2 micronutrients and its public health significance in humans is lacking. Research should focus on understanding this interaction in the context of coexisting moderate-to-severe zinc and vitamin A deficiencies in the population. *Am J Clin Nutr* 1998;68(suppl):435S–41S.

KEY WORDS *Zinc, vitamin A, interactions, mechanisms,* liver function, retinol binding protein, alcohol dehydrogenase

INTRODUCTION

Zinc participates in the absorption, mobilization, transport, and metabolism of micronutrients, including vitamin A, most likely through its involvement in protein synthesis and cellular enzyme functions. There is also evidence that vitamin A affects zinc absorption and utilization. Thus, fluctuation in the status of one or both micronutrients may reasonably expect to alter the metabolism of the other, with functional consequences on the health of the individual. Whereas several studies have shown responses of vitamin A concentrations in plasma and liver to experimental zinc deficiency and repletion, earlier reviews found evidence of an interaction between these 2 micronutrients to be inconclusive (1, 2), mainly due to a failure of most animal studies to adequately control for the secondary effects of food and growth restriction that occur in zinc deficiency. Studies in humans suggested that zinc supplementation favored vitamin A metabolism in some tissues when zinc deficiency was secondary to conditions such as alcoholic cirrhosis and other chronic liver diseases.

Two mechanisms are most often postulated to explain a potential dependence of vitamin A on zinc. One relates to a regulatory role of zinc on vitamin A transport mediated through protein synthesis (3, 4). Zinc deficiency can depress the synthesis of retinol-binding protein (RBP) in the liver and lead to lower concentrations of RBP in the plasma. Thus, reductions in plasma holo-RBP in animals fed zinc-deficient diets compared with their pair-fed, zinc-supplemented control counterparts may be due to impaired hepatic synthesis of the transport protein (5, 6), although partial food intake and growth restriction may confound this relation (6). The other postulated mechanism is an interaction between vitamin A and zinc through the ubiquitous, oxidative conversion of retinol to retinaldehyde (retinal), a critical step in the metabolic pathway of vitamin A that is well-described in the visual cycle in the retina of the eye (7) and requires the action of a zinc-dependent retinol dehydrogenase enzyme (8–9).

This review focuses on findings related to the interaction of zinc and vitamin A that have been reported since the earlier in-depth reviews to which readers are referred (1, 2), and attempts to assess the nutritional and public health significance of this interaction.

ANIMAL STUDIES

Mechanisms postulated by which zinc may regulate vitamin A metabolism have focused on the control by zinc of RBP-mediated intercellular and intracellular transport of retinol and the role of zinc as a cofactor in the synthesis of enzymes that regulate vitamin A absorption and function. Earlier studies showed that the

[1] From the Center for Human Nutrition and the Department of International Health, Johns Hopkins School of Public Health, Baltimore.

[2] Prepared under Cooperative Agreement no. DAN 0045-A-00-5094-00 between the Office of Health and Nutrition, US Agency for International Development, Washington, DC, and the Center for Human Nutrition, Dana Center for Preventive Ophthalmology, the Johns Hopkins University, Baltimore.

[3] Supported by Task Force Sight and Life (Roche, Basel, Switzerland); the United Nations Children's Fund, Nepal; and National Institutes of Health grant no. RR04060.

[4] Address reprint requests to P Christian, Division of Human Nutrition, 615 North Wolfe Street, Room 2041, Baltimore, MD 21205. E-mail: pchristi@jhsph.edu.

The American Society for Clinical Nutrition Inc. does not endorse any commercial enterprise.

188

hepatic RBP synthesis required for mobilization of retinol from the liver is dependent on adequate zinc nutriture (1, 5). More recent experiments in rats also showed cellular hepatic RBP (cRBP) concentrations to be significantly reduced (by 50%) in zinc-deficient compared with pair-fed groups with adequate zinc intake in addition to having a lower serum retinol concentration (10). The findings suggest that zinc deficiency can impair transport of retinol to organelles within a cell and, thus, alter vitamin A metabolism before impairing transport of retinol via plasma RBP. However, the finding that control animals gained more weight than zinc-deficient animals despite pair feeding makes it difficult to isolate the effects of zinc deficiency per se from effects of the accompanying generalized malnutrition and protein deficiency on cRBP concentrations.

Another mechanism for the interaction between zinc and vitamin A was examined in an experiment in pair-fed rats on the changes in retinyl ester hydrolase and acyl CoA:retinol *O*-acyltransferase, hepatic enzymes that regulate retinyl ester hydrolysis as well as alcohol dehydrogenase (ADH) and retinal oxidase, which are necessary for the conversion of retinol to retinal and retinal to retinoic acid, respectively. Zinc deficiency did not change the retinyl ester hydrolase and acyl CoA:retinol *O*-acyltransferase activities. However, ADH activity declined significantly and retinal oxidase activity increased significantly in the zinc-deficient state (11). These changes in hepatic enzyme concentrations were accompanied by an increased concentration and total content of hepatic vitamin A, offering an alternative mechanism, other than possible reduction in RBP, for decreased metabolism of retinol in zinc deficiency. Increased retinal oxidase activity may be a compensatory response to maintain normal production of retinoic acid in the presence of decreased retinol degradation to its aldehyde. However, zinc-deficient rats also gained less weight than pair-fed control animals, again making it difficult to isolate the specific effects of zinc deficiency from general malnutrition and reduced growth in producing these enzyme changes.

Zinc may affect absorption of vitamin A. Zinc deficiency reduced lymphatic uptake of retinol in rats, which was attributed to a decrease in lymphatic phospholipid output resulting from impaired biliary secretion into the intestinal lumen (12). Enterocytes of zinc-deficient rats in another experiment failed to form chylomicrons, the principal carriers of dietary lipids including retinyl esters. Adding essential fatty acids to the diet did not counteract the adverse effect of zinc deficiency (13). These studies carefully controlled food intake, feeding patterns, and body weights in zinc-deficient, pair-fed control rats, suggesting that intestinal vitamin A malabsorption could be due to specific effects of zinc deficiency beyond any generalized effects of malnutrition.

Changes in vitamin A intake have effects on zinc absorption, status, and function. In situ experiments in vitamin-deficient chicks showed that zinc absorption was depressed not only throughout the small intestine (by 40%), but particularly in the ileum (by 57%) (14). Administration of retinyl acetate increased ileal absorption of zinc nearly 3-fold. Vitamin A–dependent synthesis of a protein in the ileal mucosa, which is putatively involved in binding zinc, was identified. It must be noted that experimental chicks manifested severe hypovitaminosis A; their body weight was 60% of that of the controls and they had secondary zinc deficiency. A dramatic decline in zinc absorption is likely to occur in severe vitamin A deficiency, although the effects on zinc absorption of milder vitamin A deficiency remains unknown. In chicks fed a vitamin A–deficient diet, plasma zinc concentrations were lower (2.1 compared with 1.5 μg Zn/10^6 red blood cells) and hepatic zinc concentrations were higher (≈51 compared with 62 μg Zn/g liver) than vitamin A–adequate controls, effects that appeared to have been mediated through effects of vitamin A depletion on the activity of hepatic zinc–containing enzymes such as ADH, other hydrolytic enzymes, and superoxide dismutase (15).

Interaction in pregnancy

The singular and joint effects of maternal zinc and vitamin A deficiencies on vitamin A status and pregnancy outcomes were examined in rats fed 3 different concentrations of zinc (100, 9, and 0.5 μg Zn/g) and vitamin A (400, 8, and 0 μg·kg body $wt^{-1}\cdot d^{-1}$), representing adequate, marginally deficient, and deficient dietary concentrations, respectively, by using a factorial design (16). Marginal and deficient intakes of either zinc or vitamin A in dams and fetuses lowered plasma vitamin A concentrations compared with dams receiving adequate intakes of each nutrient. However, plasma retinol was lowest when vitamin A deficiency was accompanied by marginal-to-deficient concentrations of zinc in the diet, for which a strong statistical interaction was reported. There was also a tendency for hepatic vitamin A concentrations to increase with decreased dietary zinc, presumably reflecting impaired mobilization. Effects were observed in reproductive health outcomes. That is, the number of both implantation sites affected and malformed fetuses increased with marginal and deficient intakes of either vitamin A or zinc, but the consequences were more severe with combined deficiencies, also reflected by a statistical interaction between zinc and vitamin A with respect to both of these outcomes. However, only marginal and deficient intakes of zinc caused both maternal and fetal weight to decline, raising concern that dietary restriction and inanition may have been partly responsible for associated changes in vitamin A status and pregnancy outcomes. Subsequently, Peters et al (17), using a similar design and dietary zinc protocol, showed that supplemental dietary vitamin A given at high doses cannot ameliorate the deleterious effects of zinc deficiency on vitamin A metabolism during pregnancy. Pregnant rats were fed diets containing 100, 4.5, or 0.5 μg Zn/g food and 4 (normal) or 8 (supplemental) μg retinyl acetate/g. Maternal plasma vitamin A concentrations decreased and liver vitamin A concentrations increased with zinc deficiency, which is indicative of decreased hepatic mobilization of vitamin A possibly from a lower rate of zinc-dependent RBP synthesis. Fetuses of zinc-deficient dams also had lower concentrations of plasma and liver vitamin A than control animals fed zinc-adequate diets. However, low intake of zinc in the diet also depressed maternal food intake, weight gain of dams, and fetal and placental weight gain and increased the incidence of birth defects, all influences that could partly obscure specific effects of zinc deficiency on maternal and fetal vitamin A status.

A study in pregnant rhesus monkeys suggested that marginal zinc deficiency may alter vitamin A metabolism by altering the formation or release of holo-RBP, or both, from the liver (18). Monkeys were fed either a zinc-sufficient (100 μg Zn/g) or a zinc-deficient (4 μg Zn/g) diet. Control animals were pair fed to the zinc-deficient group but were fed 100 μg Zn/g. There was no difference in the circulating vitamin A and RBP concentrations of zinc-deficient and pair-fed pregnant controls during the third

trimester. Neither was there a significant correlation between plasma concentrations of either vitamin A or RBP and zinc in the pair-fed and ad libitum–fed control groups. However, there was a strong correlation between plasma concentrations of vitamin A (**Figure 1**) and RBP with plasma zinc in the zinc-deficient groups, both at 135 d of pregnancy and at 3 mo postpartum. The authors concluded that above a certain threshold of plasma zinc, vitamin A transport is not dependent on plasma zinc concentration, but below that threshold vitamin A release and transport from the liver is strongly influenced by plasma zinc concentration. A polynomial regression equation that included a squared term for zinc provided the optimum fit for the curvilinear relation between plasma zinc and vitamin A (18). Similarly, a polynomial equation adequately fit the observed curvilinear relation between the maternal ratio of RBP to vitamin A and plasma zinc concentration, suggesting that the lowering effect of zinc deficiency on circulating retinol is mediated by reduced RBP synthesis and release from the liver.

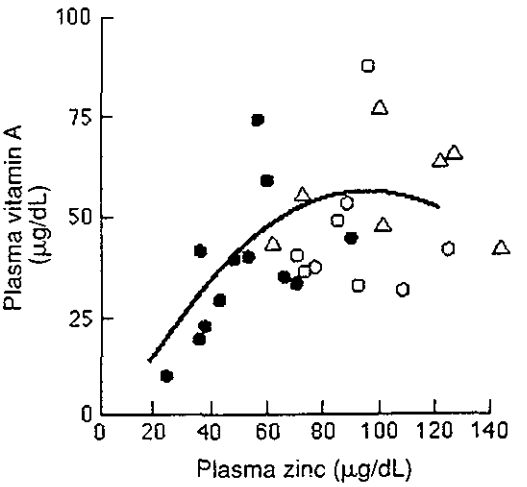


FIGURE 1. Relation between plasma vitamin A and zinc concentrations in rhesus monkeys at 135 d of pregnancy. ●, zinc-deficient; ○, food-restricted controls; △, ad libitum–fed controls. Conversion factors: for vitamin A, 1 μg/dL = 0.035 μmol/L; for zinc, 1 μg/dL = 0.153 μmol/L. Adapted from reference 18.

Interaction via the visual cycle

Animal data show that vitamin A and zinc interact during the conversion of retinol to retinal in the retina of the eye during the visual cycle (7), as well as in other tissues such as the liver and testes (9). The zinc metalloenzyme ADH is required for this oxidative process. Animal experiments and in vitro studies show that the retina is sensitive to zinc nutriture and that zinc deficiency can impair photoreceptor function by down-regulating ADH activity (8). Rhodopsin, a photosensitive pigment required for night vision, is synthesized from retinaldehyde and a membrane protein moiety, opsin. In zinc deficiency the formation of 13-*cis*-retinal is reduced, causing a decrease in rhodopsin formation and rod photosensitivity that can lead to poor dark adaptation or night blindness.

One study has questioned the role of impaired ADH activity in zinc deficiency–induced abnormal dark adaptation (19). Investigators measured the rate of rhodopsin regeneration in the following groups of rats: *1*) zinc sufficient and ad libitum fed, *2*) zinc deficient, and *3*) zinc sufficient, pair fed, and weight matched. The initial rate of rhodopsin regeneration after 60 min of dark-adaptation, using the ocular concentration of 13-*cis*-retinal as an indicator, was not different among the 3 groups. After 120 min, the rhodopsin concentration was significantly higher in the ad libitum–fed group, but not in the zinc-deficient and pair-fed groups. These results did not suggest that zinc deficiency had caused the presumed reduction in ADH activity. Rather, generalized malnutrition that results from markedly reduced food intake may have depressed opsin synthesis (although unassessed) in the rod photoreceptor cells, which in turn may have lowered rhodopsin concentrations in the eyes.

Another study that examined electroretinogram changes in zinc and vitamin A deficiencies in rats showed that vitamin A deficiency could cause retinal degeneration that was not responsive to zinc (20). Vitamin A repletion, however, reversed the degeneration even in the presence of moderate zinc deficiency. At first, rats were deprived of both zinc and vitamin A. During a second phase they were randomized to receive either a vitamin A–deficient, zinc-deficient, or zinc- and vitamin A–sufficient diet. Retinograms of the zinc- and vitamin A–sufficient and zinc-deficient groups after 60 d were nearly normal, whereas the those of the vitamin A–deficient group showed deterioration, suggesting that retinal degeneration could be reversed with vitamin A but not with zinc. The zinc- and vitamin A–sufficient group showed the greatest functional improvement but never reached the same level of function as a group of normal, continuously replete controls, indicating that some irreparable retinal damage occurred during the initial zinc- and vitamin A–depletion period.

HUMAN STUDIES

Armed with evidence that zinc deficiency could affect vitamin A status and function (eg, by altering ADH-catalyzed conversions to intermediate retinoid forms or by reducing RBP synthesis), investigators studied interactions between zinc and vitamin A in humans, involving a variety of status, growth, and health indicators, in cross-sectional studies and single- or double-nutrient interventions (**Table 1**).

Cross-sectional studies have often failed to establish a consistent relation between vitamin A and zinc status; however, a positive association may exist in malnourished populations in which deficiencies of both micronutrients often coexist. In African children suffering from measles, serum zinc was significantly correlated with both serum vitamin A ($r = 0.6$) and RBP ($r = 0.7$) (25). Mean serum zinc concentrations were lower in school-aged Bangladeshi children with serum retinol concentrations <0.7 μmol/L compared with those with concentrations >0.7 μmol/L (26). A study of 3–9-y-old American children with delayed cognition observed a significantly lower serum vitamin A concentration in children with low serum zinc concentrations (<10.7 μmol/L) than in children with higher circulating zinc concentrations (0.56 compared with 0.73 μmol/L) (23). On the other hand, no correlation was found between serum zinc and vitamin A concentrations in 102 growth-retarded (third percentile for height, weight, or head circumference) Mexican American migrant children (21). Children exhibiting normal growth were not included in the analysis. Also, no significant difference was observed in serum vitamin A concentration among pregnant teenagers of Mexican descent with low com-

190

TABLE 1
Studies of zinc–vitamin A interactions in human populations[1]

Reference and year	Population	Interaction	Comment
Cross-sectional studies of association			
Chase et al (21), 1980	Mexican American migrant children <70 mo of age	No	No significant correlation between serum vitamin A and plasma zinc.
Hunt et al (22), 1985	Pregnant teenagers of Mexican descent	No	No differences observed in serum vitamin A concentrations between subjects with low and normal zinc status
Kozlowski et al (23), 1987	US children 3–9 y of age with delayed cognition	Yes	Serum RBP and vitamin A lower among children with serum zinc <10.7 μmol/L
Dorea et al (24), 1988	Fetuses and infants who died in the first 16 wk of life	No	Weak correlation between liver zinc and vitamin A concentrations
Coutsoudis et al (25), 1990	African children with measles	Yes	Baseline serum zinc concentrations correlated with vitamin A ($r = 0.6$) and RBP ($r = 0.7$)
Ahmed et al (26), 1993	Bangladeshi children 5–12 y of age	Yes	Mean serum zinc concentrations lower among those with vitamin A concentrations <0.7 μmol/L
Supplementation trials			
Hunt et al (22), 1985	Low-income, pregnant teenagers of Mexican descent	No	Supplementation with zinc did not improve vitamin A status; maybe zinc was not limiting
Hustead et al (27), 1988	Preterm infants, birth weight <2000 g	Yes	Retinol and RBP values increased in zinc-supplemented group
Marinho et al (28), 1991	Brazilian children 3–7 y of age	Unknown	Data were not adequately compared
Udomkesmalee et al (29), 1992	Thai children 6–13 y of age	Weak support	Zinc supplementation did not improve vitamin A or RBP but improved dark adaptation time; vitamin A alone or with zinc did not improve vision but normalized conjunctival epithelium
Jalla et al (30), 1997	Indian children 5–36 mo of age	No	No effect of zinc on vitamin A status when given in addition to vitamin A

[1] RBP, retinol binding protein.

pared with acceptable zinc concentrations (22).

The few observational studies done to date are inconclusive with respect to a zinc–vitamin A status association during pregnancy. In Brazil, only a very weak and nonsignificant correlation was observed between liver zinc and vitamin A content of fetuses and stillborn infants ($r = 0.2$) or infants who died in the first 4 mo of life ($r = 0.14$) (24). In contrast, amniotic fluid and maternal serum concentrations of zinc and vitamin A were more highly correlated ($r = 0.47$ and $r = 0.32$, respectively) in 106 British mothers, among whom were 12 women whose pregnancies were terminated for the presence of neural tube defects (31).

Supplementation intervention trials might be expected to clarify the presence and strength of an interaction between zinc and vitamin A. Hunt et al (22) randomly assigned pregnant Mexican teenagers to receive vitamin-mineral supplements with and without 20 mg Zn. Serum retinol concentrations, measured initially and later in pregnancy, did not change in either the zinc or placebo groups, indicating no effect of zinc supplementation on the vitamin A status of these girls. Serum retinol was relatively high in both groups (mean of ≈1.85 μmol/L), and it is unlikely that zinc status was limiting the maintenance of vitamin A status in this population. In another study, parasitized and nonparasitized children 3–7 y of age were randomly assigned to receive daily supplements of zinc (5 mg), vitamin A, both vitamin A and zinc, or placebo for 30 d (28). The daily dosage of vitamin A was specified erroneously as "500 mg" and may be 500 μg retinyl acetate instead. Retinol concentration was measured before and after supplementation. Serum retinol improved in all 4 groups without parasites, including the placebo group, making it difficult to infer any effect on serum retinol concentrations from either zinc or vitamin A supplementation. Serum retinol remained unchanged in all 4 groups with parasitic infection. In a group of 24 American preterm infants (with birth weight <2000 g), randomized intravenous zinc supplementation increased plasma retinol (by 0.32 μmol/L, $P<0.005$) and RBP (by 8 mg/L, $P<0.1$) over changes observed in placebo-treated control subjects, suggesting that zinc may have enhanced hepatic release of vitamin A through increased RBP production (27).

In **Table 2** are summarized the findings of one of the few population-based, double-masked, randomized trials examining the interaction between vitamin A and zinc supplementation in 6–13-y-old Thai children with marginal zinc and vitamin A deficiencies (29). Children were randomly assigned to receive either 25 mg Zn, 1500 retinol equivalents (RE) vitamin A, both zinc and

TABLE 2
Effect of vitamin A and zinc supplementation on vitamin A status of Thai schoolchildren[1]

Treatment	Serum vitamin A	VRT	CIC
Vitamin A	↑	—	↑
Zinc	—	↑	↑
Vitamin A with zinc	↑	—	↑
Placebo	—	—	—

[1] VRT, visual restoration time; CIC, epithelial changes on the conjunctival surface obtained by using impression cytology; small arrows denote significant improvement ($P < 0.05$); large arrows denote stronger significance ($P < 0.01$); $n = 32–35$/group. Data from reference 29.

vitamin A, or placebo daily for 6 mo. Outcome measures included serum retinol, zinc, and RBP concentrations, visual restoration time (VRT, a dark adaptation test), and epithelial changes on the conjunctival surface obtained by impression cytology (CIC). Whereas serum retinol concentration increased with vitamin A supplementation, zinc alone did not increase circulating concentrations of either vitamin A or RBP. VRT improved slightly in the group receiving zinc but not in any other group, indicating that zinc was more limiting than vitamin A with respect to scotopic vision in this population. Although the proportion of children with abnormal CIC results decreased significantly with either zinc or vitamin A, the conjunctival surface normalized in all initially CIC-abnormal children who received both zinc and vitamin A, revealing a possible synergism between zinc and vitamin A. Although only children with marginal zinc and vitamin A deficiencies were included in the trial, a lag time of 6–16 wk between screening and baseline assessment for enrollment showed an improvement in the circulating zinc and vitamin A concentrations in the subjects, which was attributed to seasonal increases in the availability of dietary zinc sources (29), although regression to the mean was also a likely factor. Thus, at entry into the trial, subjects had zinc and vitamin A statuses comparable with those of a normal population of US children.

A double-masked, randomized trial was carried out in India to evaluate the effects of daily zinc supplementation for 4 mo on the severity and duration of diarrheal morbidity in children 6–35 mo of age, all of whom were also provided a large dose of vitamin A at the outset (32). This design made it possible to examine the effect of zinc supplementation over that of vitamin A alone (control group) on serum retinol concentration. Findings showed that by the end of the trial, mean plasma retinol concentrations were similar in both groups (0.76 μmol/L in the zinc-supplemented compared with 0.79 in the control group) (32) and comparable percentages of children in both groups had retinol concentrations <0.35 μmol/L (38% compared with 43%, respectively) (**Table 3**). Fifteen percent of children receiving vitamin A and zinc, but only 44% of those receiving vitamin A alone, had plasma retinol values of 0.35–0.70 μmol/L. Conversely, a higher proportion of children receiving vitamin A alone had plasma retinol concentrations >1.05 μmol/L compared with those receiving zinc with vitamin A (15% compared with 7%). These complex shifts in plasma retinol indicated that there was little or no effect of zinc supplements on vitamin A status of the children. It is likely that increased zinc intake may only affect vitamin A status when children are moderately to severely protein-energy deficient, as has been observed before (33), or when children have low zinc status at the outset, but this needs to be examined.

TABLE 3
Effect on plasma vitamin A concentrations of supplementing children 6–35 mo of age with vitamin A and with zinc compared with vitamin A with placebo[1]

Plasma vitamin A concentration (μmol/L)	Vitamin A with zinc	Vitamin A with placebo
≤0.35	4 (4.4)	3 (3.2)
0.35–0.7	30 (33.3)	36 (38.3)
0.7–1.05	50 (56.6)	41 (43.6)
>1.05	6 (6.7)	14 (14.9)

[1] *n* (%). Chi-square = 4.694, $P = 0.261$. Data from reference 30 and S Sazawal, R Black, unpublished observations, 1996.

ZINC–VITAMIN A INTERACTION IN DISEASE STATES

There may be an interaction between zinc and vitamin A in patients suffering from various pathologic conditions that severely compromise hepatic function such as alcoholic cirrhosis, cystic fibrosis, and idiopathic hemochromatosis. The nature and previous evidence of this interaction were reviewed by Solomons and Russell (2). Evidence of an interaction in the liver during chronic, and often wasting, disease states may provide clues to relevant interactions between these 2 nutrients in malnourished and ill populations.

The metabolism and transport of both zinc and vitamin A appear to be affected by chronic ethanol consumption and by the functional damage of hepatic cirrhosis. Several reports show an impairment in dark adaptation in alcoholic cirrhosis associated with deficiencies of zinc or vitamin A , or both (34–37). Liver cirrhosis can cause hypogonadism [which is also observed in zinc deficiency (38)], possibly because of depressed activity of zinc-dependent ADH that can also lead to lower testicular metabolism of retinol (9). Mean serum zinc and RBP concentrations were shown to be significantly lower in Nigerian cirrhotic patients with hypogonadism than in those without this condition (38). In alcoholics in Sri Lanka, serum concentrations of zinc and vitamin A were lower than in healthy control subjects (39). Serum zinc and vitamin A were positively correlated, but only in those with cirrhosis.

The presence of a zinc–vitamin A interaction was also explored in patients with cystic fibrosis, rheumatoid arthritis, and cancer of the larynx. Although Palin et al (40) showed no change in plasma vitamin A or RBP concentrations with zinc treatment in patients with cystic fibrosis, Navarro and Desquilbet (41) found that RBP was significantly lower in patients with cystic fibrosis and was positively correlated with plasma vitamin A concentrations. However, improved protein nutriture as a result of zinc supplementation could have caused the increase in plasma vitamin A concentrations (42). These studies regarding zinc-related vitamin A deficiency in cystic fibrosis are inconclusive.

Serum zinc concentrations were found to correlate positively with vitamin A and RBP concentrations in patients with cancer of the larynx, but not in patients with other nonmalignant laryngeal diseases (43). It was postulated that a rapidly growing tumor can increase the body's requirement for zinc, causing a reduction in the circulating concentrations of zinc that could depress zinc-dependent hepatic RBP synthesis and, consequently, lower plasma retinol. A similar explanation has been offered for the hypovitaminosis A observed in patients with rheumatoid arthritis (44). Associations between circulating zinc and vitamin A concentrations in disease states may reflect direct effects of one nutrient (or its relative absence) on the other; however, concurrent deficiencies of other nutrients, especially protein, may also explain these apparent interactions.

CONCLUSION

Absorption, metabolism, hepatic release, transport, and tissue utilization of vitamin A may depend, in part, on adequate zinc status. Circulating and hepatic concentrations of retinol have been observed to decline and rise in experimental zinc deficiency and repletion, respectively, in animals fed adequate amounts of vitamin A, although effects of food restriction have often not been adequately controlled for. In **Figure 2** the potential mecha-

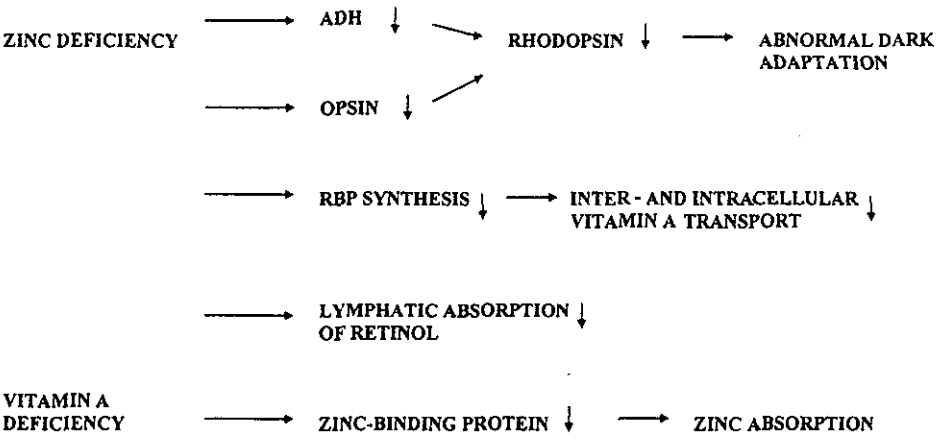


FIGURE 2. Potential mechanism for zinc and vitamin A interaction. ADH, alcohol dehydrogenase; RBP, retinol binding protein.

nisms whereby zinc and vitamin A may interact are summarized. Zinc appears to regulate the metabolic conversion of retinol to retinal, an enzymatic step requiring zinc-dependent ADH, in the intestine, liver, testes, and in the retina, where it participates in an essential step of the visual cycle. Zinc deficiency may also impair synthesis of the protein opsin. Zinc is required for hepatic synthesis of cRBP and RBP, implying a regulatory role for zinc in mobilizing vitamin A within cells and from the liver. Conversely, severe vitamin A deficiency may reduce absorption and lymphatic transport of zinc by altering synthesis of a zinc-dependent binding protein. In mammals and humans, circulating zinc and vitamin A concentrations appear unrelated in well-nourished states but tend to co-vary in marginally nourished individuals with coexisting zinc and vitamin A deficiencies.

These observations suggest that zinc deficiency could both precipitate health consequences associated with both zinc deficiency and, through its gatekeeping roles, impose a secondary vitamin A deficiency in human populations. Furthermore, zinc supplementation of marginally nourished groups might be expected to improve indicators of both zinc and vitamin A status and associated health outcomes. Zinc deficiency might also be expected to limit the health and nutritional effect of vitamin A interventions, eg, on the occurrence of night blindness in a population. Although vitamin A deficiency could also interfere with zinc efficacy, data are more sparse to support this interaction. These, however, remain theoretical concerns. Clear evidence of amplified health consequences resulting from joint deficiencies or restricted health and nutritional benefits of vitamin A in the absence of zinc interventions in humans is lacking. The few trials conducted in malnourished populations show inconsistent responses in indicators of vitamin A or zinc status when the other nutrient is introduced. Further, vitamin A interventions in the absence of zinc supplementation were highly effective in dramatically reducing night blindness and other forms of xerophthalmia, anemia, the severity of infectious morbidity, and mortality rates of young children (45) in populations in which zinc deficiency could have been expected to be prevalent. However, this does not rule out even greater potential effects of vitamin A in the presence of adequate zinc nutriture. Lack of evidence about the public health effect of a potential zinc–vitamin A interaction on health and disease, given the critical roles played by each individual nutrient in maintaining resistance to infection, should stimulate greater effort to address this issue in the future.

REFERENCES

1. Smith JC Jr. The vitamin A–zinc connection: a review. Ann N Y Acad Sci 1980;355:62–74.
2. Solomons NW, Russell RM. The interaction of vitamin A and zinc: implications for human nutrition. Am J Clin Nutr 1980;33:2031–40.
3. Mejia LA. Vitamin A–nutrient interrelationships. In: Bauernfeind JC, ed. Nutrition basics and applied science—a series of monographs. New York: Academic Press Inc, 1986:89–100.
4. Terhune MW, Sandstead HH. Decreased RNA polymerase activity in mammalian zinc deficiency. Science 1972;177:68–9.
5. Smith JE, Brown ED, Smith JC Jr. The effect of zinc deficiency on the metabolism of retinol binding protein in the rat. J Lab Clin Med 1974;84:692–7.
6. Smith JC Jr, Brown ED, McDaniel EG, Chan W. Alterations in vitamin A metabolism during zinc deficiency and food and growth restriction. J Nutr 1976;106:569–74.
7. Wald G. The interconversion of the retinenes and vitamin A in vitro. Biochem Biophys Acta 1950;4:215–28.
8. Huber AM, Gershoff SN. Effects of zinc deficiency on the oxidation of retinol and ethanol in rats. J Nutr 1975;105:1486–90.
9. Sundaresan PR, Cope FO, Smith JC Jr. Influence of zinc deficiency on retinal reductase and oxidase activities in rat liver and testes. J Nutr 1977;107:2189–97.
10. Mobarhan S, Greenberg B, Mehta R, Friedman H, Barch D. Zinc deficiency reduces hepatic cellular retinol-binding protein in rats. Int J Vit Nutr Res 1992;62:148–54.
11. Boron B, Hupert J, Barch DH, et al. Effect of zinc deficiency on hepatic enzymes regulating vitamin A status. J Nutr 1988;118:995–1001.
12. Ahn J, Koo SI. Effects of zinc and essential fatty acid deficiencies on the lymphatic absorption of vitamin A and secretion of phospholipids. J Nutr Biochem 1995;6:595–603.
13. Ahn J, Koo SI. Intraduodenal phosphatidycholine infusion restores the lymphatic absorption of vitamin A and oleic acid in zinc-deficient rats. J Nutr Biochem 1995;6:604–12.
14. Berzin NI, Bauman VK. Vitamin A-dependent zinc-binding protein and intestinal absorption of zinc in chicks. Br J Nutr 1987; 57:255–68.
15. Sklan D, Halevy O, Donoghue S. The effect of different dietary levels of vitamin A on metabolism of copper, iron and zinc in the chick. Int J Vitam Nutr Res 1987;57:11–8.

16. Duncan JR, Hurley LS. An interaction between zinc and vitamin A in pregnant and fetal rats. J Nutr 1978;108:1431–8.
17. Peters AJ, Keen CL, Lönnerdal B, Hurley LS. Zinc–vitamin A interaction in pregnant and fetal rats: supplemental vitamin A does not prevent zinc-deficiency-induced teratogenesis. J Nutr 1986;116: 1765–71.
18. Baly DL, Golub MS, Gershwin ME, Hurley LS. Studies of marginal zinc deprivation in rhesus monkeys. III. Effects on vitamin A metabolism. Am J Clin Nutr 1984;40:199–207.
19. Dorea JG, Olson JA. The rate of rhodopsin regeneration in the bleached eyes of zinc-deficient rats in the dark. J Nutr 1986; 116:121–7.
20. Kraft SP, Parker JA, Matuk Y, Venkatrao A. The rat electroretinogram in combined zinc and vitamin A deficiency. Invest Ophthalmol Vis Sci 1987;28:975–84.
21. Chase HP, Hambidge M, Barnett SE, Houts-Jacobs MJ, Lenz K, Gillespie J. Low vitamin A and zinc concentrations in Mexican-American migrant children with growth retardation. Am J Clin Nutr 1980;33:2346–9.
22. Hunt IF, Murphy NJ, Cleaver AE, et al. Zinc supplementation during pregnancy in low-income teenagers of Mexican descent: effects on selected blood constituents and on progress and outcome of pregnancy. Am J Clin Nutr 1985;42:815–28.
23. Kozlowski BW, Taylor ML, Baer MT, Blyler EM, Trahms C. Anticonvulsant medication use and circulating levels of total thyroxine, retinol binding protein, and vitamin A in children with delayed cognitive development. Am J Clin Nutr 1987;46:360–8.
24. Dorea JG, De Araújo MOG. Zinc and vitamin A in liver of fetuses and infants. Acta Pediatr Scand 1988;77:85–8.
25. Coutsoudis A, Coovadia HM, Broughton M, Salisbury RT, Elson I. Micronutrient utilization during measles treated with vitamin A or placebo. Int J Vitam Nutr Res 1991;61:199–204.
26. Ahmed F, Barua S, Mohiduzzaman M, et al. Interactions between growth and nutrient status in school-age children of urban Bangladesh. Am J Clin Nutr 1993;58:334–8.
27. Hustead VA, Greger JL, Gutcher GR. Zinc supplementation and plasma concentration of vitamin A in preterm infants. Am J Clin Nutr 1988;47:1017–21.
28. Marinho HA, Shrimpton R, Giugliano R, Burini RC. Influence of enteral parasites on the blood vitamin A levels in preschool children orally supplemented with retinol and/or zinc. Eur J Clin Nutr 1991;45:539–44.
29. Udomkesmalee E, Dhanamitta S, Sirisinha S, et al. Effect of vitamin A and zinc supplementation on the nutriture of children in Northeast Thailand. Am J Clin Nutr 1992;56:50–7.
30. Jalla S, Sazawal S, Black RE, Mazumder S, Sinha A, Bhan MK. Effect of zinc supplementation on anemia and vitamin A status in children. FASEB J 1997;11:A654 (abstr).
31. Parkinson CE, Tan JCY. Vitamin A concentration in amniotic fluid and maternal serum related to neural-tube defects. Br J Obstet Gynaecol 1982;89:935–9.
32. Sazawal S, Black RE, Bhan MK, Bhandari N, Sinha A, Jalla S. Zinc supplementation in young children with acute diarrhea in India. N Engl J Med 1995;333:839–44.
33. Shingwekar HG, Mohanram M, Reddy V. Effect of zinc supplementation on plasma levels of vitamin A and retinol-binding protein in malnourished children. Clin Chim Acta 1979;93:97–100.
34. Patek AJ, Haig C. The occurrence of abnormal dark adaptation and its relation to vitamin A metabolism in patients with cirrhosis of the liver. J Clin Invest 1939;18:609–16.
35. Russell RM, Morrison SA, Smith FR, Oaks EV, Carney EA. Vitamin A reversal of abnormal dark adaptation in cirrhosis. Ann Intern Med 1978;88:622–6.
36. Morrison SA, Russell RM, Carney EA, Oaks EV. Zinc deficiency: a cause of abnormal dark adaptation in cirrhotics. Am J Clin Nutr 1978;31:276–81.
37. McClain CJ, Van Thiel DH, Parker S, Badzin LK, Gilbert H. Alterations in zinc, vitamin A, and retinol-binding protein in chronic alcoholics. A possible mechanism for night blindness and hypogonadism. Alcohol Clin Exp Res 1979;3:135–41.
38. Abdu-Gusau K, Elegbede JA, Akanya O. Serum zinc, retinol and retinol-binding protein in levels in cirrhotics and hypogonadism. Eur J Clin Nutr 1989;43:53–7.
39. Atukorala TMS, Herath CA, Ramchandran S. Zinc and vitamin A status of alcoholics in a medical unit in Sri Lanka. Alcohol 1986;21:269–75.
40. Palin D, Underwood BA, Denning CR. The effect of oral zinc supplementation on plasma levels of vitamin A and retinol binding protein in cystic fibrosis. Am J Clin Nutr 1979;32:1253–9.
41. Navarro J, Desquilbet N. Depressed plasma vitamin A and retinol-binding protein in cystic fibrosis correlations with zinc deficiency. Am J Clin Nutr 1981;34:1439–40 (letter).
42. Underwood BA, Palin D. Reply to letter by Navarro and Desquilbet. Am J Clin Nutr 1981;34:1440 (letter).
43. Dróódó M, Gierek T, Jendryczko A, Piekarska J, Pilch J, Polanska D. Zinc, vitamin A and E, and retinol-binding protein in sera of patients with cancer of the larynx. Neoplasma 1989;36:357–62.
44. Honkanen V, Knottinen YT, Mussalo-Rauhamaa H. Vitamins A and E, retinol binding protein and zinc in rheumatoid arthritis. Clin Exp Rheumatol 1989;7:465–9.
45. Sommer A, West KP Jr. Vitamin A deficiency: health, survival, and vision. New York: Oxford University Press, 1996.

194

The American Journal of Clinical Nutrition

Official Journal of The American Society for Clinical Nutrition

9650 Rockville Pike, Bethesda, MD 20814-3998
(301) 530-7038 Fax (301) 571-8303
http://www.faseb.org/ajcn

195

The American Society for Clinical Nutrition Inc. does not endorse any commercial enterprise.

Zinc and immune function: the biological basis of altered resistance to infection[1-3]

Anuraj H Shankar and Ananda S Prasad

ABSTRACT Zinc is known to play a central role in the immune system, and zinc-deficient persons experience increased susceptibility to a variety of pathogens. The immunologic mechanisms whereby zinc modulates increased susceptibility to infection have been studied for several decades. It is clear that zinc affects multiple aspects of the immune system, from the barrier of the skin to gene regulation within lymphocytes. Zinc is crucial for normal development and function of cells mediating nonspecific immunity such as neutrophils and natural killer cells. Zinc deficiency also affects development of acquired immunity by preventing both the outgrowth and certain functions of T lymphocytes such as activation, T_h1 cytokine production, and B lymphocyte help. Likewise, B lymphocyte development and antibody production, particularly immunoglobulin G, is compromised. The macrophage, a pivotal cell in many immunologic functions, is adversely affected by zinc deficiency, which can dysregulate intracellular killing, cytokine production, and phagocytosis. The effects of zinc on these key immunologic mediators is rooted in the myriad roles for zinc in basic cellular functions such as DNA replication, RNA transcription, cell division, and cell activation. Apoptosis is potentiated by zinc deficiency. Zinc also functions as an antioxidant and can stabilize membranes. This review explores these aspects of zinc biology of the immune system and attempts to provide a biological basis for the altered host resistance to infections observed during zinc deficiency and supplementation. *Am J Clin Nutr* 1998; 68(suppl):447S–63S.

KEY WORDS Zinc, zinc deficiency, immunity, infection, micronutrients, ontogeny, apoptosis

INTRODUCTION

Animal studies in the 1930s first documented the essentiality of zinc for growth and survival of animals (1). Unfortunately, it was not until the 1960s and the seminal work of Prasad (2) that the importance of zinc deficiency in human populations was appreciated. Later, it became clear that zinc was also crucial for patients maintained on parenteral nutrition (3). A central clinical feature of zinc deficiency in these latter 2 cases was the increased susceptibility to infectious diseases. This led researchers to speculate that zinc must be important for host immunity. Indeed, the past 2 decades have witnessed a rapid growth in knowledge of the underlying mechanisms whereby zinc exerts its ubiquitous effects on immune function, disease resistance, and general health. Analysis of animal and human studies examining the in vivo effects of nutritional zinc deficiency and supplementation on immune cells and their function underscores the essential role of zinc in normal development and function of many key tissues, cells, and effectors of immunity. In vitro studies have elucidated the role of zinc at the cellular level, and recent advances in molecular biology and cell biology have begun to clarify the role of zinc in gene expression, mitosis, and apoptosis of lymphoid cells. From these studies, many of which are reviewed herein, it is clear that even mild zinc deficiency can impair multiple mediators of host immunity ranging from the physical barrier of the skin to acquired cellular and humoral immunity (4–10). This article represents a synthesis and expansion of 2 previous reviews examining the role of zinc on health and immune function (9, 10). Two figures are presented that integrate the concepts relating zinc to development and function of immune cells (**Figure 1**) and the role of zinc in the cellular processes of activation and proliferation (**Figure 2**).

ZINC DEFICIENCY

In persons suffering from marginal zinc deficiency, clinical signs are depressed immunity, impaired taste and smell, onset of night blindness, impairment of memory, and decreased spermatogenesis in males (9, 10). Severe zinc deficiency is characterized by severely depressed immune function, frequent infections, bullous pustular dermatitis, diarrhea, alopecia, and mental disturbances (3, 9). Similar effects of mild and severe zinc deficiency

[1] From the Department of International Health, Division of Human Nutrition, and Department of Molecular Microbiology and Immunology, The Johns Hopkins University School of Public Health and the Department of Internal Medicine, Division of Hematology and Oncology, Wayne State University School of Medicine, Detroit.

[2] Supported by Cooperative Agreements DAN 0045-A-00-5094-00 and HRN-A-00-97-00015-00 between the Johns Hopkins University School of Public Health and the Office of Health and Nutrition, US Agency for International Development. ASP was supported by Labcatal Laboratories, Paris, and George Eby Research, Austin, Texas.

[3] Address reprint requests to AH Shankar, Division of Human Nutrition, Room 2041, Department of International Health, The Johns Hopkins School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205. E-mail: ashankar@jhsph.edu.

196

arise in zinc-deficient laboratory animals (9–11). A rare genetic disorder, known as acrodermatitis enteropathica, occurs in cattle and humans, resulting in decreased zinc absorption accompanied by characteristic hyperpigmented skin lesions, poor growth, and low plasma zinc concentrations (12, 13).

ZINC STATUS AND SUSCEPTIBILITY TO INFECTIOUS DISEASES

Influence of zinc on experimental infections in animals

Numerous animal and human studies indicate that zinc deficiency decreases resistance to infectious diseases. Zinc-deficient animals have suppressed immune responses and are more susceptible to a diverse range of infectious agents including *Herpes simplex* virus (14) and Semliki forest virus (15); bacteria such as *Francisella tularensis* (16), *Listeria monocytogenes* (17, 18), *Salmonella enteritidis* (19), and *Mycobacterium tuberculosis* (20); the protozoan parasites *Trypanosoma cruzi* (21), *Trypanosoma musculi* (22), *Toxoplasma gondii* (23), and *Plasmodium yoelii* (24); eukaryotes such as *Candida albicans* (15, 25); and the helminths *Heligmosomoides polygyrus* (26, 27), *Strongyloides ratti* (28), *Trichinella spiralis* (29), *Fasciola hepatica* (30), and *Schistosoma mansoni* (31).

A selective overview of these studies illustrates the broad role of zinc during infection. Zinc-deficient mice infected with *T. musculi* harbored >3 times as many parasites as control mice, and this was associated with delay in production of protective antibodies (22). Likewise, 40% of moderately zinc-deficient mice succumbed to *P. yoelii* 17XNL malaria, a normally nonlethal infection (24). Again, this was accompanied by delayed appearance of protective immunoglobulin (Ig) G antibodies. With respect to *C. albicans*, genetically susceptible strains of mice were rendered resistant when fed zinc-enriched diets or when zinc was administered intraperitoneally (15, 25), and normally resistant mice became susceptible when maintained on a low-zinc diet (25). For the helminth infections, zinc-deficient animals either harbored more worms or had prolonged expulsion times. For *H. polygyrus*, marginal zinc deficiency had little effect (27), although severe zinc deficiency did increase worm burdens (26). In murine *S. mansoni*, zinc deficiency resulted in decreased pathology with no change in worm burdens (31). For *Listeria*, delayed hypersensitivity response, a measure of T lymphocyte activation, was reduced but actual bacterial clearance did not change substantially (17, 18). In naturally occurring bovine acrodermatitis enteropathica, calves had impaired resistance to viruses, fungi, and bacteria. This condition was corrected by increased dietary zinc intakes (32).

Influence of zinc on infectious diseases in human populations

Several studies have demonstrated the benefits of zinc supplementation on infectious diseases in human populations. Double-blind, placebo-controlled trials of daily zinc supplementation showed that zinc reduces the incidence and duration of acute and chronic diarrhea by 25–30% (33–35) and can reduce the incidence of acute lower respiratory infections by 45% (36). Some studies implied that zinc may reduce clinical disease caused by *Plasmodium falciparum* (37, 38), and it was recently shown in a controlled trial carried out in Papua New Guinea that zinc supplements could reduce malaria attributable to health center attendance by >35% (39). Zinc supplementation also reduced the frequency of recurrent boils in hospital patients (40). Furthermore, decreased *S. mansoni* egg counts were observed in children given zinc supplements compared with those given a placebo (41). Humans suffering from acrodermatitis enteropathica also had fewer infections when given supranormal concentrations of zinc (42), and plasma zinc concentrations were substantially lower in patients with diffuse lepromatous leprosy compared with those with the more limited bacillary form (43).

Zinc supplements also have beneficial effects when administered during infection. Zinc lozenges were shown to decrease the duration of the common cold (44, 45), and studies in preschool children showed that zinc supplementation during a diarrheal episode reduced duration of sickness up to 30% (33).

In patients with HIV, zinc deficiency is frequently seen and disease progression is accompanied by decreased serum zinc concentrations and depressed phytohemagglutinin (PHA) mitogenic responses (46, 47). These changes are partially reversible by zinc supplementation (48). Likewise, in the murine model of AIDS the amount of zinc in multiple tissues, including the spleen, was depressed (49). In contrast, recent dietary analysis of a large cohort of AIDS patients indicated that high zinc intake was associated with more rapid progression of the disease (50). The actual effect of zinc supplementation on the progression of AIDS or the general health of AIDS patients remains to be evaluated.

Direct effects of zinc on infectious agents

In general, it is not clear to what extent the availability of zinc affects in vivo growth of infectious agents themselves. Most microorganisms require zinc in some amount for basic cellular processes. For example, yeast (51), *P. falciparum* (52), and HIV-1 (53) require zinc for replication and other functions. During the acute phase response zinc is redistributed from plasma to the liver and to lymphocytes (54). It has been suggested that this is an adaptive response intended to deprive invading pathogens of zinc (54–56). However, lowered plasma zinc concentrations resulting from the acute phase response are generally well above the concentrations at which the growth of most pathogens is affected (51, 52, 55). Still, highly localized suppression of extracellular zinc to microbiostatic concentrations may be created by the acute phase response in conjunction with calprotectin, a zinc binding protein produced by polymorphonuclear leukocytes (51, 57). In contrast, it is also true that very high zinc concentrations can be microbicidal. For example, the relatively low frequency of urinary tract infections in men may be due, in part, to the very high microbicidal zinc concentration in semen (58). Moreover, the effect of zinc on the common cold may be due to the increased zinc concentrations in the nasal mucosa, which may alter the conformation of the binding site between the virus and ICAM-1 (59).

The balance between zinc availability or deficiency for host immunity and the invading pathogen is affected by multiple factors. It appears, however, that in most cases any benefit to the pathogen of zinc availability is well compensated for by the concomitant improvement in host immune function.

EFFECTS OF ZINC ON OVERALL IMMUNE FUNCTION

Zinc is a potent mediator of host resistance to infection. Because susceptibility to a broad range of pathogenic agents is affected by zinc, it is likely that multiple immunologic effectors

are involved. The general effects of zinc on innate or nonspecific and adaptive or specific immunity are presented below as a framework for the more detailed discussions of zinc and specific immunologic effectors presented later.

Effects of zinc on barrier and nonspecific immunity

Evaluation of the effects of zinc on host immunity must begin with the effect of zinc on components of innate immunity. Zinc deficiency damages epidermal cells, resulting in the characteristic skin lesions of acrodermatitis enteropathica or severe zinc deficiency (42, 60). Damage to the linings of the gastrointestinal and pulmonary tracts is also observed during zinc deficiency (9, 61). As detailed below, zinc deficiency affects other mediators of nonspecific immunity, such as polymorphonuclear leukocyte function (6, 42, 62–64), natural killer cell function (6, 65–67), and complement activity (68).

Overview of effects of zinc on specific immunity

Lymphopenia is common in zinc-deficient humans and animals and occurs in both the central and peripheral lymphoid tissues (9, 10, 69). Adult mice fed zinc-deficient diets for 2 wk had reduced numbers of T and B lymphocytes in peripheral blood and spleen tissue (69). Peripheral blood lymphocyte and macrophage concentrations were eventually reduced by >50% (6, 69). Importantly, even marginal zinc deficiency substantially suppressed peripheral blood lymphoid cell concentrations in mice and humans (61, 69–71).

Zinc deficiency results not only in decreased lymphocyte concentrations, but in depressed T and B lymphocyte function (69, 72–76). In general, murine T and B lymphocyte proliferative responses to mitogens were greatly depressed in zinc-deficient compared with pair-fed animals (77–79). Suppression ranged from 5% to 50% with a tendency for greater suppression in T lymphocytes.

Some reports in rat and murine systems, however, documented no effect of zinc deficiency on mitogenic responses of lymphocytes (80, 81). Indeed, some data suggest that the primary effect of zinc deficiency is deletion of T and B lymphocytes with little loss of function in the surviving cells. This is based on plaque-forming cell (PFC) assays measuring antibody production in vitro in which data were expressed as PFC per viable splenocytes rather than PFC per whole spleen (81). These findings notwithstanding, it is difficult to explain the effects of zinc on immunity solely on the grounds of changing cell numbers. Given the ubiquitous involvement of zinc in cellular events, ranging from gene expression to membrane stability (9, 10, 82), one would expect multiple metabolic and structural defects in the surviving lymphocytes in zinc-deficient hosts.

Effects of high-dose zinc supplementation on immune function

Elevated zinc intake has been shown in some cases to potentiate immune function above basal levels. In rats, PHA-induced mitogen responses were increased in animals fed a rich 1000-ppm Zn diet for 14 d compared with a 40-ppm control diet (83). Likewise, mice fed a high-zinc diet had increased numbers of splenic PFC in response to T lymphocyte–dependent antigens (25). Additional murine studies confirmed increased T lymphocyte and macrophage function with supplemental doses of zinc (15). Interestingly, the mice were more also more resistant to endotoxin shock (15). A high-zinc diet also diminished the amount of lipid peroxidation in the livers of mice infected with *Plasmodium berghei* (84).

Studies in humans concerning excess zinc intake on the immune system are few. One study reported that 11 men receiving 300 mg Zn/d, 20 times the recommended dietary allowance (RDA) (9), for 6 wk experienced decreased proliferative responses of lymphocytes to PHA and reductions in chemotaxis and phagocytosis of circulating polymorphonuclear leukocytes (85). Additional studies suggest that very high zinc intakes in adults and children can result in copper deficiency, anemia, growth retardation, and immunodepression (86–88). One recent study of nutritional rehabilitation in marasmic children suggested that zinc down-regulated certain monocyte functions (89). Importantly, other larger and longer-term controlled trials of high-dose zinc supplementation in adults did not induce immunosuppression (90–92). Moreover, deleterious immunologic effects were not observed in trials in which clinically healthy and otherwise normal children received daily zinc supplementation up to 2 times the RDA. Therefore, short-term intake of zinc several-fold above the RDA is generally considered safe in preschool children and adults (82, 93, 94). It should, however, be mentioned that high-dose zinc supplements given to mouse pups during the perinatal period reduced PFC responses (95), and high doses may also have adverse health effects in human neonates (94). As for any nutritional supplement, caution must be exercised in taking excessive doses of zinc for prolonged periods of time.

EFFECTS OF FETAL ZINC DEFICIENCY ON IMMUNOLOGIC DEVELOPMENT

Gestational zinc deficiency in mice and nonhuman primates has short- and long-term deleterious effects on the offspring (96–100). Substantial reductions are seen in lymphoid organ size and gamma globulin concentrations in pups born to marginally zinc-deficient mice. In rhesus monkeys, prenatal zinc deficiency led to hypogammaglobulinemia, reductions in mitogenesis of peripheral blood lymphocytes, and reduced neutrophil function (101).

Additional murine studies showed that many of the immunodeficiencies observed at birth persisted into adulthood even though the pups were fed a diet containing normal amounts of zinc after weaning. At 6 wk of age, serum IgM concentrations were only 1% of normal and IgG_{2a} and IgA concentrations remained 40–60% below normal (96–98). As expected, the PFC response to T-dependent and T-independent antigens was markedly reduced (96–98). Indirect evidence for such effects in humans is also available. Intrauterine growth retardation, which has been linked to maternal zinc deficiency (102), results in depressed cellular immunity that can persist for years (103, 104). Furthermore, nutritional deficiencies occurring up to 1 y of age have resulted in permanent reductions in thymus size (105).

The observation that prenatal zinc deficiency in mice results in long-term suppression of IgM, IgA, and IgG_{2a} concentrations suggests that a transient prenatal window may exist for the development of cells producing these antibodies. Indeed, during immunologic ontogeny, groups of certain B lymphocytes emerge in sequential waves, with each wave being idiotypically distinct, thereby recognizing specific predefined antigenic determinants (106, 107). Many of these cells produce antibodies of the IgM and IgA isotypes that recognize capsular polysaccharides of bacteria and other common pathogens (106). These so-called natural

198

antibodies are believed to provide an early line of defense for the immunologically naive neonate against invasive pathogens. Natural antibodies also influence the developing antibody repertoire of the immune system. Postnatally, the bone marrow undergoes developmental maturation and loses the potential to generate cells secreting these antibodies (108). Thus, if gestational zinc deficiency influences the development of cells producing such antibodies, the antibody repertoire to bacterial antigens would be permanently altered in adults having experienced transient gestational zinc deprivation.

To test this hypothesis, we examined the antibody responses to the common bacterial antigens α-1,3-dextran and phosphorylcholine in adult mice whose mothers had been moderately deprived of zinc during late gestation. Adult mice who had been subjected to transient fetal zinc deficiency in utero had similar concentrations of total serum IgM, but more than 2-fold greater IgM responses to phosphorylcholine and up to 50% lower responses to α-1,3 dextran compared with control animals (AH Shankar, unpublished observations, 1998). The data suggest that transient zinc deficiency in utero permanently altered the response to certain bacterial antigens. Moreover, whereas total IgM concentrations were similar in both sets of animals, the findings indicate that antibody repertoire is more sensitive to mild or transient zinc deficiency in utero.

The potential consequences of altered antibody diversity can be seen in immune responses to 2 common childhood respiratory pathogens, *Streptococcus pneumoniae* and *Haemophilus influenza*. During infection with *S. pneumoniae*, children often mount a potent antibody response. However, only certain antibodies are protective, and their absence can result in severe infection or death (107, 109). Likewise, in children it has been shown that among the antibodies produced against *H. influenza*, those of the Hib-id1 idiotype have the greatest antibacterial activity (110).

Zinc status may also affect placental transport of antibodies from the mother to the fetus during the last trimester of pregnancy. In contrast with natural antibodies, these maternal antibodies recognize a greater diversity of antigens with high specificity. However, they are not replenished after birth and gradually decay to low values by 6 mo of age. In addition to providing early protection, maternal antibodies also influence qualities of the emerging immune system in the child (108). Because zinc is important for normal placental development (111, 112), deficiency may result in impaired in utero acquisition of maternal antibodies by the child as well. The actual effect of gestational or perinatal zinc deficiency on transfer of maternal antibodies to the child in utero or through breast milk in unknown.

Perhaps the most remarkable effects of gestational zinc deficiency is the demonstration in mice that some immunodeficiencies, particularly suppressed IgM concentrations, persisted to the second- and third-generation offspring (96). This suggests that gestational zinc deficiency may have epigenetic effects (113). Given the potential short- and long-term immunologic consequences of even moderate gestational zinc deficiency, a better understanding of its influence on public health is clearly needed.

INFLUENCE OF ZINC ON IMMUNOSUPPRESSIVE CONDITIONS

Persons suffering from sickle cell anemia have depressed peripheral T lymphocyte numbers, decreased $CD4^+$-$CD8^+$ T lymphocyte ratios, loss of delayed hypersensitivity, decreased natural killer cell activity, decreased production of the T lymphocyte cytokine interleukin (IL)-2, and suppressed activity of thymulin, a thymic hormone (4, 70, 114, 115). Zinc supplementation restored these immunologic indexes to near normal (69, 116). Likewise, in patients with Down syndrome, zinc supplements restored immediate hypersensitivity, normalized in vitro assays of lymphocyte function and neutrophil chemotaxis (117), and increased resistance to infection (118). Zinc supplements also restored delayed hypersensitivity in alcoholics (119) and stimulated cell-mediated and humoral immunity in humans and mice with hypogammaglobulinemia (25, 120).

In elderly humans and animals, impairments in wound healing and resistance to infection were corrected by zinc supplementation (121–124), suggesting that immunodeficiency in the elderly is due in part to zinc deficiency. Low plasma zinc concentrations in elderly patients were associated with peripheral blood lymphopenia, although not with depressed serum IgA concentrations or delayed hypersensitivity responses (125). Zinc supplementation restored normal lymphocyte and neutrophil zinc concentrations, increased concentrations of circulating T lymphocytes, and improved delayed hypersensitivity and IgG antibody responses to tetanus toxoid. As might be expected, increased resistance to infection was also observed (122, 126–130).

EFFECTS OF ZINC ON SPECIFIC CELLS OF THE IMMUNE SYSTEM

As discussed above, zinc deficiency has multiple effects on the immune system. The reader is referred to Figure 1 as a guide to the various effects of zinc deficiency in the context of overall immunologic development and function.

T lymphocytes

Development of T lymphocytes

Studies of zinc deficiency in bovine (131, 132), porcine (133, 134), rat (135), and murine (5, 6, 73, 77, 136) systems, and in severely zinc-deficient children (7, 137–140), describe substantial reductions in the size of the thymus, the central organ for T lymphocyte development. Mice maintained on a zinc-deficient diet for as little as 2 wk showed moderate thymic involution. After 4 wk the thymus retained only 25% of its original size, and at 6 wk only a few thymocytes remained in the thymic capsule (6). The percentage of thymic atrophy exceeded that of other organs and the percentage of overall weight loss. The reduction in thymic size and cellularity was observed mostly in the thymic cortex, where immature thymocytes develop. Such changes were not observed in pair-fed animals, confirming that zinc was responsible for the effect (6, 73, 77). After only 1 wk of normal zinc intake, thymic size increased and the cortex was repopulated with cells (73). In contrast with the effects of severe zinc restriction on thymic size in adult mice, moderate zinc restriction had minimal effects on thymic size (5). However, marginal zinc deficiency in the early postnatal period did result in substantial reductions in thymic size (77).

In the peripheral lymphoid organs, T lymphocytes were progressively depleted from the spleen, lymph nodes, and peripheral blood in zinc-deficient animals (6, 69, 73). Zinc-deficient children with acrodermatitis enteropathica or patients receiving total parenteral nutrition have reduced numbers of lymphocytes, particularly T lymphocytes in the blood and peripheral lymphoid tis-

sues. Decreased $CD4^+$:$CD8^+$ cell ratios are also seen. Recent studies in an experimental human model showed that the percentage of $CD8^+CD73^+$ T lymphocytes, precursors to cytotoxic T lymphocytes, was decreased in zinc deficiency (141). The presence of the CD73 molecule on cytotoxic T lymphocytes is required for antigen recognition, proliferation, and cytolysis (141). These and the previously mentioned changes are reversed with zinc supplementation (7, 32, 65, 138, 141–144).

T lymphocyte function

T lymphocyte responses such as delayed hypersensitivity and cytotoxic activity are suppressed during zinc deficiency and reversed by zinc supplementation (6, 7, 32, 137, 142, 144, 145). Suppressed delayed hypersensitivity responses in malnourished children are also restored after zinc supplementation (139, 140, 146, 147). In one study in children, synergism between zinc and vitamin A tended to potentiate the T-lymphocyte proliferative response to tuberculin (145). Patients receiving total parenteral nutrition devoid of zinc had reduced T lymphocyte PHA responses (65, 142, 143), which returned to normal after 20 d of zinc supplementation. The influence of zinc on T-dependent antibody production and T lymphocyte cytokine production patterns are discussed below in the sections titled B lymphocytes and cytokines, respectively.

In vitro effects of zinc on T lymphocytes

Several in vitro studies showed that zinc is required for T lymphocyte proliferation in response to IL-1 (148), PHA, concanavalin A, or IL-2 (149–152). Proliferation of human T lymphocytes was enhanced by zinc when stimulated with mitogenic concentrations of PHA, concanavalin A, or phorbol ester (153). Moreover, the mitogen response of cells from zinc-deficient patients could be improved by addition of 10 μmol $ZnCl_2$/L in vitro. Zinc also restored proliferative T lymphocyte responses in the presence of high concanavalin A concentrations, 20 μg/mL, which are known to be suppressive. One murine study found no effect when zinc chloride was added to spleen cell cultures containing subactivating concentrations of PHA or concanavalin A (154). In contrast, zinc potentiated the suppression of T lymphocyte mitogenesis caused by phorbol ester pretreatment (155). In another experimental system, zinc suppressed the allogeneic proliferative response of human T lymphocytes to HeLa cells (153).

It was also shown that zinc can potentiate the response to some bacterial superantigens by facilitating binding between the superantigen and class II molecules on antigen-presenting cells (156, 157). The clinical significance of this phenomenon, including the role of zinc in the development of toxic shock during sepsis, requires further study.

Lastly, there is also evidence that zinc addition in vitro alters the expression, function, or both, of lymphocyte surface molecules governing cell–cell interactions. The addition of zinc chloride at μmol/L levels enhanced the rosetting of peripheral blood T lymphocytes with sheep erythrocytes. This effect was mediated at the T lymphocyte level because pretreatment of the lymphocytes themselves, but not the sheep erythrocytes, had the same effect (158). It was also reported that zinc enhanced the transcription and expression of the adhesion molecule ICAM-1 on the surface of lymphoid cells, but not on fibroblasts (159).

The in vitro studies make apparent the many roles played by zinc in T lymphocyte activation and proliferation. The effects of zinc on T lymphocytes are modulated by factors such as the antigen presenting cell type, adhesion molecules, co-stimulatory molecules, antigen type, and the overall milieu in which T lymphocyte activation occurs.

B lymphocytes

Development of B lymphocytes

B lymphocyte development in bone marrow is adversely affected by zinc deficiency (160–162). When mice were fed a marginally zinc-deficient diet for just 30 d, nucleated bone marrow cells were reduced by one third with preferential reduction in small nongranular cells. Total B lymphocytes and their precursors were reduced nearly 75%. Losses were predominantly in pre-B and immature B lymphocytes, which declined by ≈50% and 25%, respectively. Mature B lymphocytes were less affected. This finding is consistent with the previously mentioned study wherein spleen cells remaining in zinc-deficient mice appeared functionally normal (81) with typical markers of T and B lymphocyte maturation (163). Thus, zinc deficiency blocks development of B lymphocytes in the marrow, resulting in fewer B lymphocytes in the spleen.

B lymphocyte function

B lymphocyte antibody responses are inhibited by zinc deficiency (5, 73, 164, 165). As previously mentioned, gross effects of zinc deficiency on B lymphocyte mitogen and plaque-forming responses have been observed. Zinc is required for the B lymphocyte mitogenic and cytokine response to lipopolysaccharide (156, 166). In vitro antibody production, determined by PFC assay, was strongly inhibited in splenic B lymphocytes from zinc-deficient mice (72).

Interestingly, T-dependent antibody responses are more affected by zinc deficiency than are T-independent ones. The PFC responses to the T-dependent antigen sheep red blood cells and T-independent antigen dextran were reduced by 90% (72, 73) and 50% (69, 79, 167), respectively, in zinc-deficient mice. Similar differences in effects were seen in moderately zinc-deficient animals (72, 73). In 1 study, mild zinc deficiency affected antibody responses to T-dependent antigens but not T-independent antigens (71). When zinc-deficient mice were returned to a normal diet, IgM PFC activity recovered within 1 wk, although restoration of IgG PFC activity required 1 mo, suggesting that greater perturbations in B or T lymphocyte functions are required for isotype switching (73, 164). T lymphocyte–mediated effects of zinc deficiency on B lymphocyte activity were also shown by restoration of PFC responses after infusion of normal thymocytes into zinc-deficient mice (72). In zinc-deficient mice infected with *P. yoelii*, antiparasite IgM concentrations were not affected, although IgG concentrations were suppressed nearly 10-fold (AH Shankar, unpublished observations, 1998). These findings are consistent with the idea that T-dependent B lymphocyte functions are more affected than T-independent ones. This is further supported by the observation that B lymphocytes are less dependent on zinc for proliferation than are T lymphocytes (168, 169).

It is also important to note that zinc-deficient mice had reduced antibody recall responses to T-dependent and T-independent antigens for which they had previously been immunized (69, 164, 165, 170), implying that immunologic memory is affected by zinc status. The potentially important effect of this for maintenance of vaccine efficacy in humans remains unexplored.

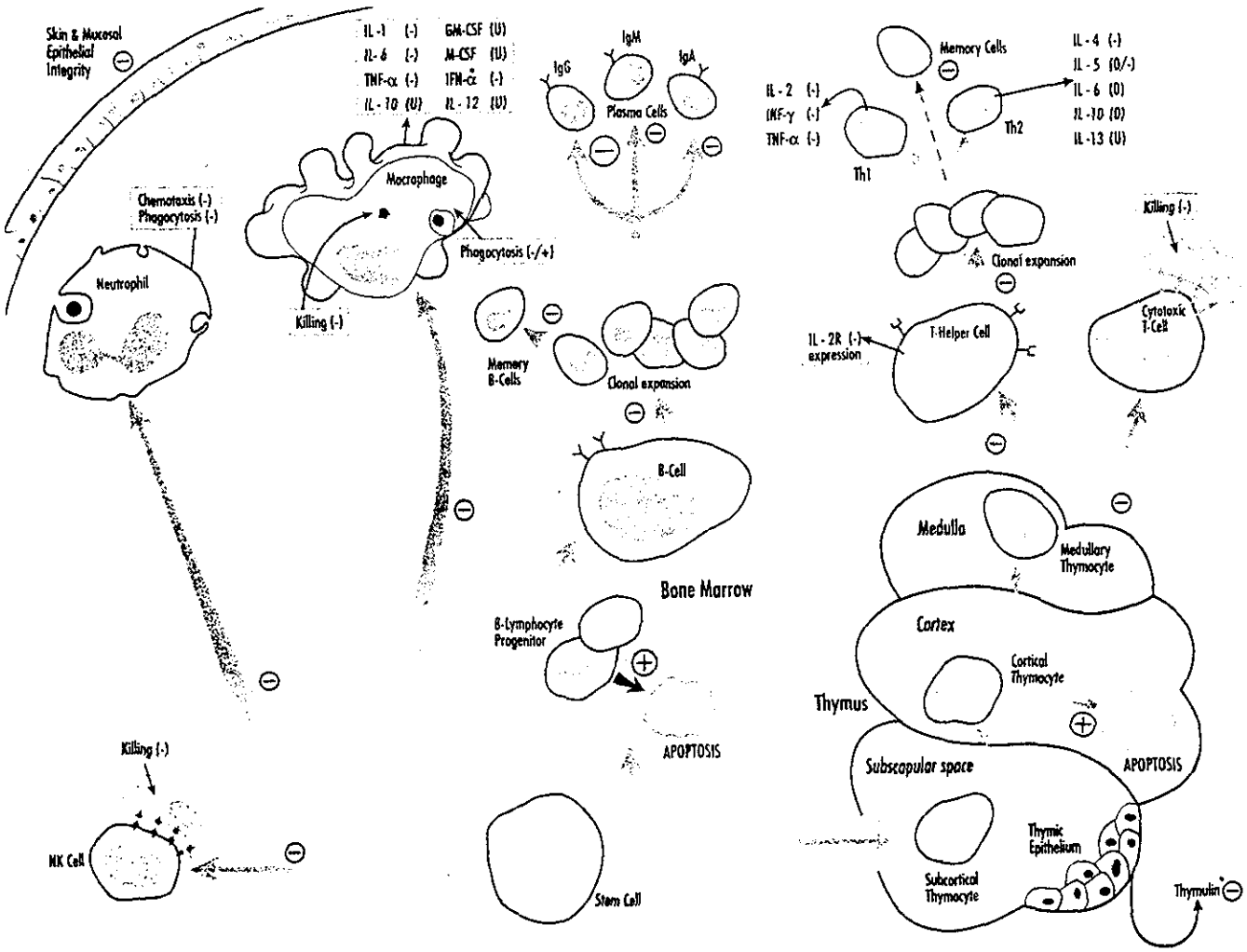


FIGURE 1. The effects of zinc on the development and function of certain immunologic cells and cytokines. GM-CSF, granulocyte macrophage colony stimulating factor; IG, immunoglobulin; IFN, interferon; IL, interleukin; IL-2R, interleukin-2 receptor; M-CSF, monocyte colony stimulating factor; NK, natural killer; 0, zinc deficiency has little or no effect on the process or activity; TNF, tumor necrosis factor; U, the effect of zinc deficiency on the particular process or activity is unknown; ⊖, zinc deficiency down-regulates or inhibits the process or activity; ⊕, zinc deficiency enhances the process or activity; *, zinc is needed for the structural integrity of the molecule.

In vitro effects of zinc on B lymphocytes

As for T lymphocytes, in vitro zinc-induced polyclonal activation of human B lymphocytes from blood, spleen, and lymph nodes was reported (171). These effects may, however, be secondary to zinc activation of helper T lymphocytes. In contrast with the effects on T lymphocytes, rosette formation between murine B lymphocytes and erythrocytes was inhibited when B lymphocytes were precultured with zinc in the presence of zinc ionophore, indicating that receptor down-regulation had occurred (172).

Natural killer cells

Studies in humans and animals describe decreased natural killer cell activity in zinc deficient states (6, 65), although 1 study reported increased natural killer cell activity (66). Natural killer cell function was depressed after in vivo treatment of cells with 1,10-phenanthroline, a zinc chelator, and the depression was reversed by the readdition of zinc, but not of calcium or magnesium (65). Exogenous zinc also stimulated production of interferon γ by human peripheral blood natural killer cells (67, 173). However, exposure of natural killer cells to high concentrations of zinc in vitro inhibited cytotoxicity by rendering target cells more resistant to damage (66, 174). This and other reports of zinc-mediated inhibition (66) of natural killer cell activity may be partially explained by the recent demonstration that the killer cell inhibitory receptor requires zinc (175). The zinc-induced down-regulation of CD16, one of the Fcγ antibody receptor subtypes on natural killer cells, may also be due to a zinc-dependent metalloprotease (176). Overall, the in vitro data are consistent with the decreased natural killer cell activity seen in zinc-deficient animals and humans (6, 61, 65).

Neutrophils

Polymorphonuclear leukocyte function is altered in zinc-deficient animals and patients with acrodermatitis enteropathica and other types of zinc deficiency. In most cases, absolute numbers of peripheral polymorphonuclear leukocytes were not affected, but chemotactic responses were impaired and were reversible by in vitro addition of zinc (42, 62, 63). In addition, in vitro addi-

201

tion of zinc potentiated the polymorphonuclear leukocyte response against staphylococcal bacteria (177). One study observed that exercise-induced potentiation of polymorphonuclear leukocyte microbicidal superoxide formation was attenuated by zinc supplementation (64). Unfortunately, the effects of zinc deficiency on microbicidal functions such as total oxygen production, phagocytosis, and extravasation remain to be adequately evaluated.

Monocytes and macrophages

Effects on monocyte and macrophage function have been observed during zinc deficiency (178–181). In humans, the chemotactic response of monocytes from acrodermatitis enteropathica patients is suppressed and can be restored after addition of zinc in vitro (42, 62). Monocytes from zinc-deficient mice have impaired killing of intracellular parasites, which is rapidly corrected in vitro by addition of zinc (178). Reduced macrophage phagocytosis of *Candida* has also been observed in zinc-deficient animals (15, 25). In other studies, however, the ability of macrophages from zinc-deficient rodents to phagocytose particles was either enhanced (179, 182) and accompanied by greater numbers of cells bearing Fcγ and complement receptors (179), or remained unchanged (183). High concentrations of zinc in vitro inhibited macrophage activation, mobility, phagocytosis, and oxygen consumption (65, 184, 185). When marasmic children were rehabilitated with a zinc-containing regimen, monocyte phagocytic and fungicidal activity was suppressed (89). Because elevated zinc concentrations can inhibit complement activation (68), complement-mediated phagocytosis may be adversely affected by high zinc concentrations. Additional studies are clearly needed for a better understanding of the conditions under which zinc affects monocyte and macrophage phagocytosis.

There is much speculation regarding the role of zinc in the killing of pathogens by oxygen radicals produced by macrophages. With the exception of 1 report (186), available data do not provide evidence linking zinc status with macrophage production of oxygen radicals (21, 165, 180). Unfortunately, data regarding the effects of zinc on the production of other microbicidal radicals, ie, nitric oxide, are lacking. It was proposed that the product of the *Nramp* gene in mice, responsible for resistance to mycobacteria, *Leishmania donovani*, and *Salmonella typhimurium* (187), may be a zinc transporter involved in nitric oxide–mediated microbicidal activity of macrophages. In humans, monocyte production of cytokines IL-1β, IL-6, interferon α (IFN-α) and tumor necrosis factor α (TNF-α), were stimulated by addition of zinc in vitro (188–190). However, zinc deficiency enhanced human monocyte-mediated cytotoxicity against target cells, which returned to normal after zinc supplementation (65).

Zinc deficiency suppressed macrophage support of mitogen-induced T lymphocyte proliferation (181). Although inhibition of mitogenesis was due to effects on both macrophages and T lymphocytes, suppression of macrophage function occurred earlier in the onset of zinc deficiency. Moreover, macrophage activity was corrected within 30 min of incubation with zinc salts in vitro. The rapid restoration of certain macrophage functions after zinc addition suggests that rapid therapeutic effects of zinc supplementation on diarrhea or the common cold may involve some aspects of macrophage function. Moreover, there may be greater benefit of zinc supplementation for infections in which specific macrophage functions are central to resistance. Unfortunately, there is limited information concerning the effects of zinc on microbicidal or immunologic functions of macrophages in humans, including phagocytosis, free radical production, antigen processing, antigen presentation, and cytokine production.

EFFECTS OF ZINC STATUS ON SOLUBLE MEDIATORS OF IMMUNITY

Glucocorticoids

The release of glucocorticoid hormones from the adrenal glands can result in certain physiologic changes, such as thymic atrophy, that are reminiscent of zinc-deficiency (191–193). Because zinc deficiency raises blood glucocorticoid concentrations (194–196), thymic atrophy may be mediated, in part, by glucocorticoids. Indeed, when adrenalectomized mice were fed a zinc-deficient diet for up to 6 wk, changes in thymic weight were small or absent (196). In addition, when adult mice were given a slow-release corticosteroid implant, thymus size was reduced >than 80% (161). Steroid-implanted mice also showed large reductions in pre-B and immature B lymphocytes in bone marrow (161, 197), suggesting that the effects of zinc deficiency on early B lymphocyte development may also involve glucocorticoids.

The contribution of glucocorticoids to the effects of zinc deficiency must, however, be interpreted with caution. Zinc deficiency has profound effects on human thymocytes, which are relatively resistant to glucocorticoids (196). In addition, although the thymus of zinc-deficient adrenalectomized mice remained normal in size, zinc-dependent decreases in the cortical to medullary area still occurred (196). Likewise, in adrenalectomized mice, zinc deficiency reduced IgM and IgG PFC responses to sheep red blood cells, 50% of the loss in T lymphocyte helper function in control animals occurred before detectable increases in plasma corticosterone (196). Last, in marginally zinc-deficient mice, loss of lymphocytes in the spleen, depressed immunity, and decreased IL-2 production were observed despite the absence of thymic shrinkage or increased glucocorticoid concentrations (69, 72).

Thymulin

Thymulin is a 9-peptide hormone (Glu-Ala-Lys-Ser-Gln-Gly-Ser-Asn) secreted by thymic epithelial cells. Thymulin binds to high-affinity receptors on T lymphocytes and promotes T lymphocyte maturation, cytotoxicity, and IL-2 production (198). Zinc is bound to thymulin in a 1:1 stoichiometry via the side chains of asparagine and the hydroxyl groups of the 2 serines (199). Thymulin activity, in vitro and in vivo in both animals and humans, is dependent on plasma zinc concentrations such that marginal changes in zinc intake or availability affect thymulin activity (32, 70, 120, 200–203). Thymulin is readily detectable in the serum of zinc-deficient patients, but is not active (70). The binding of zinc results in a conformational change that produces the active form of thymulin (200). The overall role of thymulin on the immunologic lesions caused by zinc deficiency has not been well studied. The use of thymulin as an indicator of zinc deficiency has been suggested, although thymulin concentration is also modulated by zinc-independent factors. Assays regarding zinc status and thymulin zinc saturation may prove more useful, much as the index of transferrin saturation provides useful information regarding iron status.

Cytokines

Cytokines, also known as interleukins, are key messengers of immunologic cells that regulate multiple aspects of leukocyte

biology. Their effects are mediated through corresponding receptors on target cells. Production or biological activity of multiple cytokines (IL-1, IL-2, IL-3, IL-4, IL-6, IFN-γ, IFN-α, TNF-α, and migration inhibitory factor) influencing the development and function of T lymphocytes, B lymphocytes, macrophages, and natural killer cells is affected by zinc deficiency. The cytokines IL-1 (204), IL-2 (71, 76, 204), IL-4 (26, 188), and IFN-γ (67) have been reported to be suppressed during zinc deficiency. A decline in IL-2 receptor expression has been observed in some (76, 152), but not all (71), studies. The amino acid sequence of IL-3 contains an active zinc binding site that is important for activity (205). Further studies are needed to determine the in vivo role of zinc in modulating IL-3 activity. Zinc also plays a critical role in the dimerization of IFN-α necessary for its activity (206). Confirming the general inhibitory effects of zinc deficiency on cytokine production in vivo, in vitro addition of 1,10-phenanthroline reversibly inhibited antigen-stimulated production of migration inhibitory factor from lymphocytes (20, 25, 207). As previously mentioned, monocyte production of cytokines IL-1β, IL-6, IFN-α, and TNF-α was stimulated by the addition of zinc in vitro (188–190, 204).

T helper cells can be categorized as Th1 and Th2 cells, according to their functions in cell-mediated (Th1) and antibody-mediated (Th2) immunity. These subsets are well characterized in the murine immune system, where IL-2, IFN-γ, and TNF-α are considered products of Th1 cells, and IL-4, IL-5, IL-6, IL-10, and IL-13 are products of Th2 cells (208). Although not as clear cut in humans, segregated T lymphocyte production of cytokines has been observed (208). Th1 cell–associated cytokines are known to promote macrophage activation and production of cytophilic IgG isotypes. Th2 cell–associated cytokines tend to suppress macrophage function and cell-mediated immunity while promoting production of the noncytophilic IgG isotypes and IgE (208).

In an experimental human model, we showed recently that the production of IFN-γ was decreased whereas the production of IL-4, IL-6, and IL-10 was not affected during zinc deficiency (209). Earlier studies in experimental human model subjects and in patients with sickle cell disease and head and neck cancer showed a significant effect of zinc deficiency on IL-2 activity (70, 210). In summary, the studies showed that even mild zinc deficiency in humans may be accompanied by an imbalance of Th1 cell and Th2 cell function, resulting in dysregulated resistance to infection.

CELL BIOLOGY OF ZINC IN THE IMMUNE SYSTEM

General cell biology of zinc

Zinc is present in the body almost exclusively as Zn^{2+} bound to cellular proteins. Zinc has a high affinity for electrons, enabling interactions with several amino acid side chains. Interactions, especially with sulfur and nitrogen atoms in the amino acids cysteine and histidine, respectively, enable zinc to cross-link remote regions within and between polypeptides to modify tertiary protein structure and function. Given this ability, its relative nontoxicity, and the fact that it does not engage in damaging redox reactions, zinc is ideally suited to play a central role in intracellular metabolism.

The zinc content within lymphocytes, 150 pmol/10^6 peripheral blood lymphocytes (211), is typical of most cells in the body. It is thought that activated lymphocytes take up zinc via multiple mechanisms, including receptors for zinc-transferrin, albumin, α2-macroglobulin, and metallothionein, the major zinc binding protein of the body (9, 149, 212–214)

Zinc uptake also involves other less well characterized mechanisms such as anionic channels or transporters (215). Recently, zinc transporters and channels mediating both the inward and outward movement of zinc were isolated from mammalian cells (216, 217). The continued study of these transporters and channels will help elucidate the mechanisms of zinc homeostasis within cells. In rats, a system for transport of zinc into the nucleus is suggested by the rapid appearance of ^{65}Zn in the nucleus of spleen cells within 2 h of intragastric feeding with ^{65}Zn (218).

Role of zinc in the cell cycle of lymphocytes

When lymphocytes were treated with PHA or concanavalin A, within several hours there was a rapid increase in cellular zinc (10, 219–221). These findings are consistent with studies indicating a requirement for zinc during mid to late G1 phase in promotion of thymidine kinase expression (222), and in another less well defined step involved in cell transition to S phase (223). The zinc-dependent activity of DNA polymerase may account in part for the influence of zinc during S phase (224). Zinc may also play a role in transition to the G2 and M phases. A greater proportion of S compared with G2 phase cells were observed in PHA-stimulated lymphocytes from mildly zinc-deficient patients suffering from sickle cell anemia (116). The ratio returned to normal after a period of zinc supplementation (116). The M phase of the cell cycle may also be affected by zinc deficiency because defective tubulin polymerization is seen in tissues from zinc-deficient animals (225, 226), and zinc is known to bind the amino-terminal of tubulin, thereby stabilizing microtubule formation (225, 226).

Mechanisms underlying the effect of zinc on cell replication

Zinc influences the activity of multiple enzymes at the basic levels of replication and transcription, as shown in Figure 2. These include DNA polymerase, thymidine kinase, DNA-dependent RNA polymerase, terminal deoxyribonucleotidyl transferase and aminoacyl transfer RNA synthetase (220, 222, 227–231), and the family of transcriptional regulators known as zinc finger DNA binding proteins (232, 233). In addition, zinc forms the active enzymatic sites of many metalloproteases (232, 233).

The activity of the major enzyme regulating DNA replication, DNA polymerase, is zinc dependent. It is inhibited by zinc deficiency and zinc chelators and is enhanced by addition of low concentrations of zinc in vitro (229). Thymidine kinase, crucial for phosphorylated pyrimidines, is also very sensitive to dietary zinc depletion (219, 229). Zinc is, in fact, required for expression of multiple genes regulating mitosis, including thymidine kinase, ornithine decarboxylase, and *CMYC* (219, 234, 235). As mentioned, several transcription factors, such as NFκ-B (236), metallothionein transcription factor 1 (237), and RING (238), contain zinc finger–like domains that are influenced by changes in intracellular pools of zinc. In addition, zinc deprivation affects the activity of RNA polymerase (230) needed for transcription.

Role of zinc in lymphocyte activation

Zinc also plays a role in multiple aspects of T lymphocyte activation and signal transduction (239) as detailed in Figure 2. Zinc is implicated in the noncovalent interaction of the cytoplas-

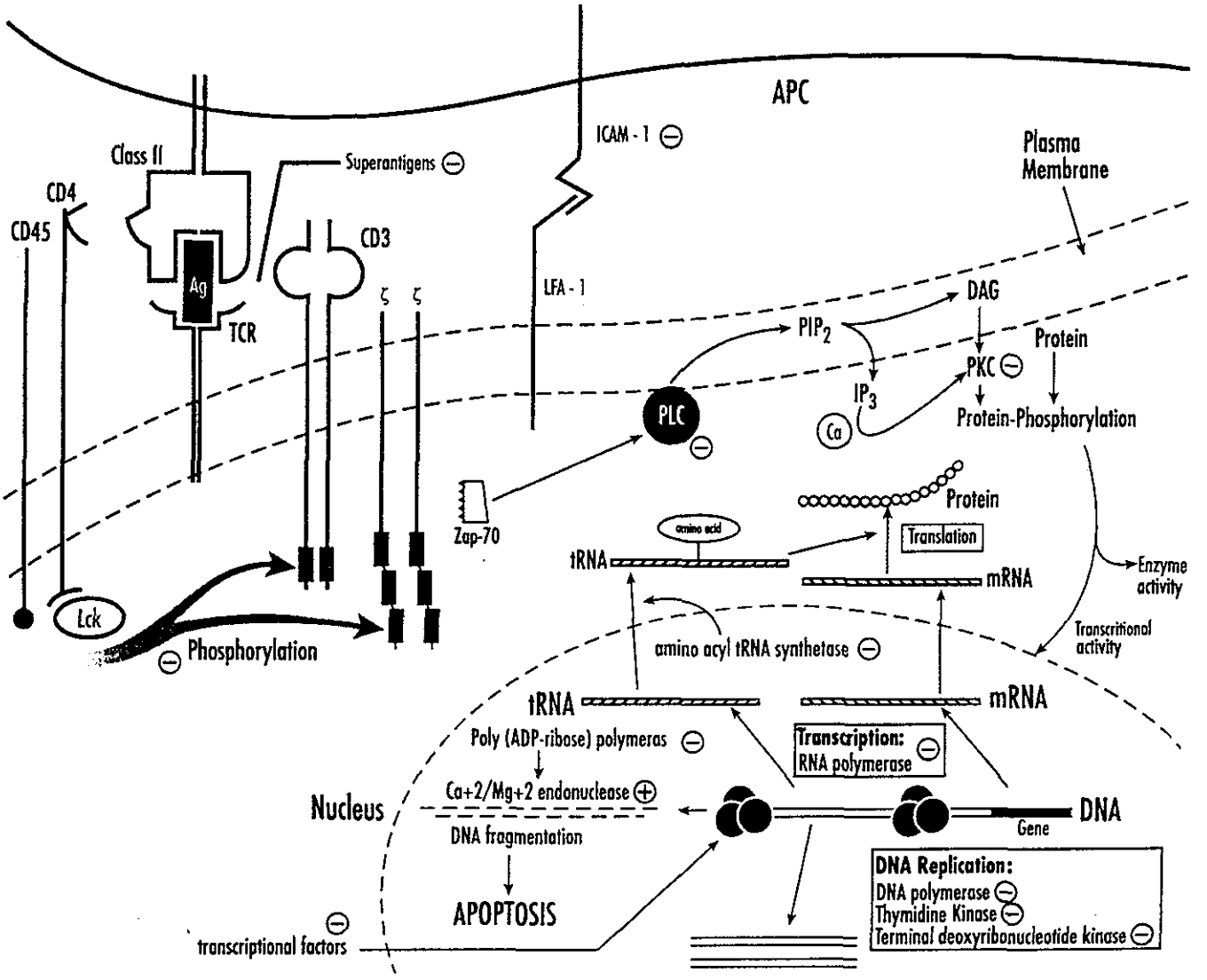


FIGURE 2. The influence of zinc deficiency on activation of lymphocytes. T lymphocyte activation via antigen presentation by class II or via superantigen binding directly to the T lymphocyte receptor (TCR). Ag, antigenic peptide; APC, antigen presenting cell; Lck, $p56^{lck}$; PLC, phospholipase C; PIP_2, phosphatidyl inositol; IP_3, inositol triphosphate; DAG, diacylglycerol, PKC, protein kinase C; ⊖indicates that zinc deficiency down-regulates or inhibits the process or activity; ζ, ζ-chains of the CD3 T lymphocyte receptor complex; Zap-70, ζ-associated protein-70; ⊕ indicates that zinc deficiency enhances the process or activity. Cell activation pathways shown are limited to those where zinc may play a role.

mic tails of CD4 and CD8 with the tyrosine kinase $p56^{lck}$, an essential protein in the early steps of T lymphocyte activation. Through this and possibly other pathways, zinc stimulates autophosphorylation of tyrosine residues by $p56^{lck}$ and subsequent phosphorylation of the T lymphocyte receptor complex involving CD45 (240, 241). Zinc is then involved in the activity of phospholipase C to give rise to inositol triphosphate and diacylglycerol (242). In addition, zinc affects the phosphorylation of proteins mediated by protein kinase C (243). Subsequent changes through protein phosphorylation regulate activation and cell proliferation.

Influence of zinc on apoptosis

The major mechanism of cell death in the body and in culture is apoptosis, a form of cell suicide characterized by a decrease in cell volume, dramatic condensation of the chromatin and cytoplasm, and fragmentation of nuclear DNA (244). Apoptosis is a normal physiologic process enabling a variety of important processes from epithelial turnover to T and B lymphocyte development (245). The dysregulation of such a basic process would, therefore, have important health consequences.

Zinc-deficient animals exhibit enhanced spontaneous and toxin-induced apoptosis in multiple cell types (243, 246–249). As previously discussed, zinc thymic atrophy is a central feature of zinc deficiency. It is now known that this atrophy is accompanied by apoptotic cell death of thymocytes (160). Several studies showed that zinc is a regulator of lymphocyte apoptosis in vivo. Zinc supplementation decreased mycotoxin-induced apoptosis of macrophages and T lymphocytes in mice (250). In addition, zinc administration to mice 48 h before intraperitoneal injection of lipopolysaccharide greatly abrogated subsequent

204

apoptotic DNA cleavage in thymocytes and loss in thymic weight (251). In vitro, greater numbers of lymphocytes and thymocytes undergo apoptosis when cultured with a zinc-free medium (252) or with zinc chelators (253–255). Conversely, apoptosis of T lymphocytes induced by in vitro exposure to toxins (250) and other agents (243) is prevented by the addition of high concentrations of zinc salts. Cells can also be rescued from apoptosis with physiologic concentrations of zinc salts (5–25 μmol/L) if uptake is facilitated by the zinc ionophore pyrithione (253). It has been suggested that zinc is a major intracellular regulator of apoptosis because lymphocytes maintain intracellular zinc at concentrations slightly above those needed to suppress apoptosis (256). In addition, a dose-responsive relation is seen between intracellular zinc concentrations and the degree of susceptibility to apoptosis (10, 256).

The subcellular mechanisms whereby zinc affects apoptosis are not well understood, but it is likely that zinc acts at multiple levels. There was a good correlation between inhibition of Ca^{2+}/Mg^{2+} DNA endonuclease activity and inhibition of apoptotic DNA fragmentation (257). Although in vivo data are lacking, the Ca/Zn balance may regulate endonuclease activity (258). Nucleoside phosphorylase, another zinc-dependent enzyme, may inhibit apoptosis by preventing the accumulation of toxic nucleotides (126, 259, 260). Likewise, poly (ADP-ribose) polymerase, the zinc-dependent nuclear enzyme, interacts with and inhibits the Ca^{2+}/Mg^{2+} DNA endonuclease (261, 262). In lymphocytes undergoing apoptosis, cytoplasmic zinc increases significantly, possibly originating from release of zinc from nuclear proteins (263).

Role of zinc as an antioxidant

Zinc has other properties that could contribute to its role in lymphocyte function. It is an antioxidant, protecting cells from the damaging effects of oxygen radicals generated during immune activation (264, 265). Zinc also regulates the expression in lymphocytes of metallothionein and metallothionein-like proteins with antioxidant activity (213, 266). Membrane zinc concentrations are strongly influenced by dietary zinc deficiency and supplementation (267, 268). Zinc concentrations in cell membranes appear to be important in preserving their integrity through poorly defined mechanisms involving binding to thiolate groups (265). It is noteworthy that zinc release from thiolate bonds can prevent lipid peroxidation (269). In addition, nitric oxide induces zinc release from metallothionein, the primary zinc binding and transport protein in the body, which may limit free radical membrane damage during inflammation. Indeed, zinc supplementation has been shown to prevent pulmonary pathology due to hyperoxia in rats (270).

DISCUSSION

The studies presented herein represent the scope of zinc-mediated effects on infectious disease and immune function. Although the effects of zinc deficiency on immunity are profound and ubiquitous, it is remarkable that greater effects on health are not observed in marginally zinc-deficient populations. This may be partially explained by the redundancy of immunologic effectors compensating for suboptimal function of any one effector. However, it is also clear that not all aspects of immunity or infections are affected equally by zinc deficiency. For example, in vitro intracellular killing by macrophages is very sensitive to zinc deficiency and is restored rapidly after supplementation. T lymphocyte–macrophage interactions are less sensitive to zinc deficiency and macrophage phagocytosis is the least sensitive. Thus, infections in which macrophage killing is a central effector, such as malaria or tuberculosis, may be more sensitive to zinc deficiency. Likewise, zinc deficiency may have greater effects on infections requiring T lymphocyte–dependent antibody responses and those in which IgG is crucial for protection. Infections for which T lymphocyte–independent responses are sufficient may be less affected. In the case of T lymphocytes, infections for which Th1 cytokines, eg, IFN-γ, are needed for resistance, may be more affected by zinc deficiency. It is also clear that development of immunologic cells is most sensitive to zinc deficiency during the perinatal period, indicating that this is a crucial window of intervention.

Future directions

Continued understanding of how zinc influences the immune system and alters host resistance to infection will depend on greater integration of modern immunology, molecular biology, and cellular biochemistry into laboratory and field-based studies. A few specific areas are detailed below that represent only a small fraction of the immunologic processes for which major gaps remain in the knowledge of zinc immunobiology.

Although adhesion molecules play a major role in cell-to-cell interaction, the influence of zinc on their function remains mostly unexplored. Given the role of zinc in the tertiary structure of proteins, it is likely that zinc deficiency may affect adhesion molecule function. Likewise, we know very little about how zinc might affect lymphocyte trafficking in the body.

With regard to macrophage function, it is still not clear how zinc influences the ability of the cell to engulf and kill organisms. Additional studies examining the effects of zinc on nitric oxide and oxygen radical production would, therefore, be useful. The role of zinc in antigen digestion and presentation to T lymphocytes is unknown.

With respect to B lymphocyte function, the processes of isotype switching and affinity maturation are crucial to optimal antibody-mediated resistance to infection. Knowledge of the influence of zinc on antibody affinity would also provide information relevant to vaccine efficacy. For both T and B lymphocytes, the role of zinc in the development of memory cells is potentially important for vaccine efficacy and needs to be further explored. As indicated for macrophages, the role of zinc in the antigen processing and presentation functions of B lymphocytes is completely unknown.

It is clear that zinc plays a major role in immunologic ontogeny and development of the antigen-recognition repertoire. In addition, subsets of T and B lymphocytes known as γδ-T lymphocytes and CD5 B lymphocytes provide pseudospecific or oligoclonal immunity. Although more limited in antigenic reactivity than their conventional polyclonal T and B lymphocyte counterparts, their localization to the pleural and peritoneal cavities and intestinal epithelium provides a rapid response against invading organisms. The effects of zinc on development of these cells and their function remains unknown. As detailed elsewhere in this supplement (271), the health effects of gestational zinc deprivation are potentially far-reaching and need to be fully explored given the possible long term or even generational effects on populations.

Concerning cytokines and T lymphocyte regulation, the seminal work by Prasad et al (209, 210) concerning the influence of

zinc on Th1 and Th2 regulation provides a foundation on which to build. Little is known regarding effects of zinc on cytokine activation of cells because most studies have focused on cytokine secretion. Moreover, most of the studies of T and B lymphocytes have used mitogens for cell activation. Studies are needed that examine lymphocyte activation in response to the more physiologic antigen driven responses involving all aspects of cell adhesion, co-stimulation, and cytokine activation.

It will be important to apply knowledge of the immunologic mechanisms modulated by zinc deficiency to the design of therapies and public health interventions involving targeted zinc supplementation for certain diseases or high-risk groups. By extension, it would be useful to identify the specific immunologic lesions caused by zinc deficiency responsible for altered host resistance in natural infections and in experimental infectious disease models. The role of zinc in immunity and the biological basis of altered resistance to infection will no doubt be fruitfully explored as researchers continue to integrate knowledge and techniques from diverse disciplines to examine the effects on zinc on immunity, health, and disease.

We thank Robert Morreale for the artwork and Heather Haberly for research assistance.

REFERENCES

1. Todd WR, Elvejheim CA, Hart EB. Zinc in the nutrition of the rat. Am J Physiol 1934;107:146–56.
2. Prasad AS. Discovery of human zinc deficiency and studies in an experimental human model. Am J Clin Nutr 1991;53:403–12.
3. Kay RG, Tasman-Jones C. Acute zinc deficiency in man during intravenous alimentation. Aust N Z J Surg 1975;45:325–30.
4. Frost P, Chen JC, Rabbani I, Smith J, Prasad AS. The effect of Zn deficiency on the immune response. In: Brewer GJ, Prasad AS, ed. Zinc metabolism: current aspects in health and disease. New York: Alan R Liss Inc, 1977:143–53.
5. Luecke RW, Simonel CE, Fraker PJ. The effect of restricted dietary intake on the antibody mediated response of the zinc deficient A/J mouse. J Nutr 1978;108:881–7.
6. Fernandes G, Nair M, Onoe K, Tanaka T, Floyd R, Good RA. Impairment of cell mediated immune functions by dietary Zn deficiency in mice. Proc Natl Acad Sci U S A 1979;76:457–61.
7. Oleske JM, Westphal ML, Shore S, Gorden D, Bogden JD, Nahmias A. Zinc therapy of depressed cellular immunity in acrodermatitis enteropathica. Am J Dis Child 1979;133:915–8.
8. Good RA. Nutrition and Immunity. J Clinical Immunol 1981; 1:3–11.
9. Walsh CT, Sandstead HH, Prasad AS, Newberne PM, Fraker PJ. Zinc health effects and research priorities for the 1990's. Environ Health Perspect 1994;102:5–46.
10. Zalewski PD. Zinc and immunity: implications for growth, survival and function of lymphoid cells. J Nutr Immunol 1996;4:39–80.
11. Clegg MS, Keen CL, Hurley LS. Biochemical pathologies of zinc deficiency. In: Mills CR, ed. Zinc in human biology. New York: Springer-Verlag, 1989:129–45.
12. Barnes PM, Moynahan EJ. Zinc deficiency in acrodermatitis enteropathica: multiple dietary intolerance treated with synthetic diet. Proc R Soc Med 1973;66:327–9.
13. Neldner KH, Hambidge KM. Zinc therapy of acrodermatitis enteropathica. N Engl J Med 1975;292:879–82.
14. Feiler LS, Smolin G, Okumoto M, Condon D. Herpetic keratitis in zinc deficient rabbits. Invest Ophthalmol Vis Sci 1982;22:788–95.
15. Singh KP, Zaidi SI, Raisuddin S, Saxena AK, Murthy RC, Ray PK. Effect of zinc on immune functions and host resistance against infection and tumor challenge. Immunopharmacol Immunotoxicol 1992;14:813–40.
16. Pekarek RS, Hoagland AM, Powanda MC. Humoral and cellular immune response in zinc-deficient rats. Nutr Rep Int 1977; 16:267–76.
17. Coghlan LG, Carlomagno MA, McMurray DN. Effect of protein and zinc deficiencies on vaccine efficacy in guinea pigs following pulmonary infection with Listeria. Med Microbiol Immunol (Berl) 1988;177:255–63.
18. Carlomagno MA, Coghlan LG, McMurray DN. Chronic zinc deficiency and listeroisis in rats: acquired cellular resistance and response to vaccination. Med Microbiol Immunol (Berl) 1986; 175:271–80.
19. Kidd MT, Qureshi MA, Ferket PR, Thomas LN. Dietary zinc-methionine enhances mononuclear-phagocytic function in young turkeys. Zinc-methionine, immunity, and Salmonella. Biol Trace Elem Res 1994;42:217–29.
20. McMurray DN, Bartow RA, Mintzer CL, Hernandez-Frontera E. Micronutrient status and immune function in tuberculosis. Ann N Y Acad Sci 1990;587:59–69.
21. Fraker PJ, Caruso R, Kierszenbaum F. Alteration in the immune and nutritional status of mice by synergy between Zn deficiency and infection with *Trypanosoma cruzi*. J Nutr 1982;112:1224–9.
22. Lee CM, Humphrey PA, Aboko-Cole GE. Interaction of nutrition and infection: effect of zinc deficiency on resistance to *Trypanosoma musculi*. Int J Biochem 1983;15:841–7.
23. Tasci S, Sengil AZ, Altindis M, Arisoy K. The effect of zinc supplementation in experimentally induced *Toxoplasma gondii* infection. J Egypt Soc Parasitol 1995;25:745–51.
24. Shankar AH, Kumar N, Scott AL. Zinc-deficiency exacerbates experimental malaria infection in mice. FASEB J 1995;9:A4269 (abstr).
25. Salvin SB, Horecker BL, Pan LX, Rabin BS. The effect of dietary zinc and prothymosin a on cellular immune responses of RF/J mice. Clin Immunol Immunopathol 1987;43:281–8.
26. Shi HN, Scott ME, Stevenson MM, Koski KG. Zinc deficiency impairs T lymphocyte function in mice with primary infection of *Heligmosomoides polygyrus* (Nematoda). Parasite Immunol 1994;16:339–50.
27. Minkus TM, Koski KG, Scott ME. Marginal zinc deficiency has no effect on primary or challenge infections in mice with *Heligmosomoides polygyrus* (Nematoda). J Nutr 1992;122:570–9.
28. Fenwick PK, Aggett PJ, MacDonald DC, Huber C, Wakelin D. Zinc deprivation and zinc repletion: effect on the response of rats to infection with *Strongyloides ratti*. Am J Clin Nutr 1990;52:173–7.
29. Fenwick PK, Aggett PJ, Macdonald DC, Huber C, Wakelin D. Zinc deficiency and zinc repletion: effect on the response of rats to infection with *Trichinella spiralis*. Am J Clin Nutr 1990; 52:166–72.
30. Flagstad T, Andersen S, Nielsen K. The course of experimental *Fasciola hepatica* infection in calves with a deficient cellular immunity. Res Vet Sci 1972;13:468–75.
31. Nawar O, Akridge RE, Hassan E, el-Gazar R, Doughty BL, Kemp WM. The effect of zinc deficiency on granuloma formation, liver fibrosis, and antibody responses in experimental schistosomiasis. Am J Trop Med Hyg 1992;47:383–9.
32. Good RA. A note on zinc and immunocompetence. In: Mills CF, ed. Zinc in human biology. New York: Springer-Verlag, 1989:221–3.
33. Sazawal S, Black RE, Bhan MK, et al. Zinc supplementation reduces the incidence of persistent diarrhea and dysentery among low socio-economic children in India. J Nutr 1996;126:443–50.
34. Sazawal S, Black R, Jalla S, Bhan MK, Bhandari N, Sinha A. Zinc supplementation in young children with acute diarrhea in India. N Engl J Med 1995;333:839–44.
35. Ruel MT, Ribera JA, Santizo MC, Lonnerdal B, Brown KH. Impact of zinc supplementation on morbidity from diarrhea and respiratory infections among rural Guatemalan children. Pediatrics 1997; 99:808–13.
36. Sazawal S, Black R, Jalla S, Mazumdar S, Sinha A, Bhan MK. Zinc supplementation reduces the incidence of acute lower respiratory

206

infections in infants and preschool children: a double-blind controlled trial. Pediatrics 1998;102:1–5.
37. Gibson RS, Heywood A, Yaman C, Sohlstrom A, Thompson LU, Heywood P. Growth in children from the Wosera subdistrict, Papua New Guinea, in relation to energy and protein intakes and zinc status. Am J Clin Nutr 1991;53:782–9.
38. Bates CJ, Evans PH, Dardenne M, et al. A trial of zinc supplementation in young rural Gambian children. J Nutr 1993;69:243–55.
39. Shankar AH, Genton B, Tamja S, et al. Zinc supplementation can reduce malaria-related morbidity in preschool children. Am J Trop Med Hyg 1997;57:A434 (abstr).
40. Brody I. Treatment of recurrent furunculosis with oral zinc. Lancet 1977;2:1358 (letter).
41. Friis H, Ndhlovu P, Mduluza T, et al. The effect of zinc supplementation on *Schistosoma mansoni* reinfection rate and intensities: a randomized, controlled trail among rural Zimbabwean schoolchildren. Eur J Clin Nutr 1997;51:33–7.
42. Hambidge KM, Walravens PA, Neldner KH. The role of zinc in the pathogenesis and treatment of acrodermatitis enteropathica. In: Brewer GJ, Prasad AS, eds. Zinc metabolism: current aspects in health and disease. New York: Alan R Liss, 1977:329–40.
43. Mathur NK, Sharma M, Mangal HN, Rai SM. Serum zinc levels in subtypes of leprosy. Int J Lepr Other Mycobact Dis 1984;52:327–30.
44. Godfrey JC, Godfrey NJ, Novick SG. Zinc for treating the common cold: review of all clinical trials since 1984. Altern Ther Health Med 1996;2:63–72.
45. Mossad SB, Macknin ML, Medendorp SV, Mason P. Zinc gluconate lozenges for treating the common cold. A randomized, double-blind, placebo-controlled study. Ann Intern Med 1996; 125:81–8.
46. Bogden JD, Baker H, Frank O. Micronutrient status and human immunodeficiency virus (HIV) infection. Ann N Y Acad Sci 1990; 587:189–95.
47. Pifer LL, Wang YF, Chiang TM, Ahokas R, Woods DR, Joyner RE. Borderline immunodeficiency in male homosexuals: is life-style contributory? South Med J 1987;80:687–91, 697.
48. Falutz J, Tsoukas C, Gold P. Zinc as a cofactor in human immunodeficiency virus-induced immunosuppression. JAMA 1988;259:2850–1.
49. Wang Y, Liang B, Watson RR. The effect of alcohol consumption on nutritional status during murine AIDS. Alcohol 1994;11:273–8.
50. Tang AM, Graham NM, Saah AJ. Effects of micronutrient intake on survival in human immunodeficiency virus type 1 infection. Am J Epidemiol 1996;143:1244–56.
51. Sohnle PG, Hahn BL, Santhanagopalan V. Inhibition of *Candida albicans* growth by calprotectin in the absence of direct contact with the organisms. J Infect Dis 1996;174:1369–72.
52. Ginsberg H, Gorodetsky R, Krugliak M. The status of zinc in malaria (*Plasmodium falciparum*) infected human red blood cells: stage dependent accumulation, compartmentation and effect of dipicolinate. Biochim Biophys Acta 1986;886:337–44.
53. Bess JW, Powell PJ, Issaq HJ, et al. Tightly bound zinc in human immunodeficiency virus type 1, human T cell leukaemia virus type I and other retroviruses. J Virol 1992;66:840–7.
54. Beisel WR. Herman Award Lecture, 1995: infection-induced malnutrition—from cholera to cytokines. Am J Clin Nutr 1995; 62:813–9.
55. Clohessy PA, Golden BE. Microbiostatic activity of human plasma and its relation to zinc and iron availability. Biochem Soc Trans 1996;24:311S.
56. Sugarma B, Epps LR, Stenback WA. Zinc and bacterial adherence. Infect Immun 1982;37:1191–9.
57. Clohessy PA, Golden BE. Calprotectin-mediated zinc chelation as a biostatic mechanism in host defense. Scand J Immunol 1995; 42:551–6.
58. Fair WR, Heston WDW. The relationship of bacterial prostatitis and zinc. In: Brewer GJ, Prasad AS, eds. Zinc metabolism: current aspects in health and disease. New York: Alan R Liss, 1977: 129–40.
59. Novick SG, Godfrey JC, Godfrey NJ, Wilder HR. How does zinc modify the common cold? Clinical observations and implications regarding mechanisms of action. Med Hypotheses 1977;46:295–302.
60. Aggett RJ. Severe zinc deficiency. In: Mills CR, ed. Zinc in human biology. New York: Springer-Verlag, 1989:259–79.
61. Solomons NW. Zinc and copper. In: Shils ME, Young VR, eds. Modern nutrition in health and disease. 7th ed. Philadelphia: Lea and Febiger, 1988:238–62.
62. Weston WL, Huff JC, Humbert JR, Hambridge KN, Neldner KH, Walravens PA. Zinc correction of defective chemotaxis in acrodermatitis enteropathica. Arch Dermatol 1977;13:422–5.
63. Briggs WA, Pedersen M, Mahajan S, Sillix DH, Prasad AS, McDonald FD. Lymphocyte and granulocyte function in zinc treated and zinc deficient hemodialysis patients. Kidney Int 1982; 21:827–32.
64. Singh A, Failla ML, Deuster RA. Exercise-induced changes in immune function: effects of zinc supplementation. J Appl Physiol 1994;76:2298–303.
65. Allen JI, Perri RT, McClain CJ, Kay NE. Alterations in human natural killer cell activity and monocyte cytotoxicity induced by zinc deficiency. J Lab Clin Med 1983;102:577–89.
66. Chandra RK, Au B. Single nutrient deficiency and cell-mediated immune responses. I. Zinc. Am J Clin Nutr 1980;33:736–8.
67. Salas M, Kirchner H. Induction of interferon gamma in human leukocyte cultures stimulated by Zn^{2+}. Clin Immunol Immunopathol 1987; 45:139–42.
68. Montgomery DW, Chvapil M, Zukoski CF. Effects of zinc chloride on guinea pig complement component activity in vitro: concentration-dependent inhibition and enhancement. Infect Immunol 1979; 23:424–31.
69. Fraker PJ, Gershwin ME, Good RA, Prasad A. Interrelationships between zinc and immune function. Fed Proc 1986;45:1474–9.
70. Prasad AS, Meftah S, Abdallah J, et al. Serum thymulin in human zinc deficiency. J Clin Invest 1988;82:1202–10.
71. Moulder K, Steward MW. Experimental zinc deficiency: effects on cellular responses and the affinity of humoral antibody. Clin Exp Immunol 1989;77:269–74.
72. Fraker PJ, Haas SM, Luecke RW. Effect of zinc deficiency on the immune response of young adult A/J mice. J Nutr 1977;107:1889–95.
73. Fraker PJ, DePasquale-Jardieu R, Zwickl CM, Luecke RW. Regeneration of T-cell helper function in zinc-deficient adult mice. Proc Natl Acad Sci U S A 1978;75:5660–4.
74. Good RA, Fernandes G, Yunis EJ, et al. Nutritional deficiency, immunologic function, and disease. Am J Pathol 1976;84:599–614.
75. Cunningham-Rundles C, Cunningham-Rundles S, Iwata T, et al. Zinc deficiency, depressed thymic hormones, and T lymphocyte dysfunction in patients with hypogammaglobulinemia. Clin Immunol Immunopathol 1981;21:387–96.
76. Dowd PS, Kelleher J, Guillou PJ. T lymphocyte subsets and interleukin 2 production in zinc deficient rats. Br J Nutr 1986;55:59–69.
77. Beach RS, Gershwin ME, Hurley LS. Altered thymic structure and mitogen responsiveness in postnatally zinc-deprived mice. Dev Comp Immunol 1979;3:725–38.
78. Gross RL, Osdin N, Fong L, Newberne PM. Depressed immunological function in zinc-deprived rats as measured by mitogen response of spleen, thymus, and peripheral blood. Am J Clin Nutr 1979;32:1260–6.
79. Fraker PJ, Zwickl CM. Immune repair capacity of zinc deficient young adult and neonatal mice. Fed Proc 1981;40:918 (abstr).
80. Kramer TR. Reevaluation of zinc deficiency on concanavalin A-induced rat spleen lymphocyte proliferation. J Nutr 1984;114:953–63.
81. Cook-Mills JM, Fraker PJ. Functional capacity of the residual lymphocytes in zinc deficient mice. Br J Nutr 1993;69:835–48.
82. Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. Physiol Rev 1993;73:79–118.

83. Chvapil M, Zukoski CF, Hattler BG, et al. Zinc and cells. In: Prasad AS, Oberleas D, eds. Human health and disease: zinc and cCopper. New York: Academy Press, 1976:269–81.
84. Arif AJ, Mathur PD, Chandra S, Singh C, Sen AB. Effect of zinc diet on Xanthine oxidase activity of liver of mice infected with *Plasmodium berghei*. Indian J Malariol 1987;24:59–63.
85. Chandra RK. Excessive intake of zinc impairs immune response. JAMA 1984;252:1443–6.
86. Fosmire GJ. Zinc toxicity. Am J Clin Nutr 1990;51:225–7.
87. Prasad AS, Brewer GJ, Schoomaker EB, Rabbani P. Hypocupremia induced by zinc therapy in adults. JAMA 1978;240:2166–8.
88. Porter KG, McMaster D, Elmes ME, Love AH. Anaemia and low serum-copper during zinc therapy. Lancet 1977;2:774 (letter).
89. Schlesinger L, Arevalo M, Arredondo S, Lonnerdal B, Stekel A. Zinc supplementation impairs monocyte function. Acta Paediatr 1993;82:734–8.
90. Bogden JD, Oleske JM, Lavenhar MA, et al. Zinc and immunocompetence in elderly people: effects of zinc supplementation for 3 months. Am J Clin Nutr 1988;48:655–63.
91. Brewer GJ, Johnson V, Kaplan J. Treatment of Wilson's disease with zinc: XIV. Studies of the effect of zinc on lymphocyte function. J Lab Clin Med 1997;129:649–52.
92. Bogden JD, Oleske JM, Lavenhar MA, et al. Effects of one year supplementation with zinc and other micronutrients on cellular immunity in the elderly. J Am Coll Nutr 1990;9:214–25.
93. Sandstrom B. Consideration in estimates of requirements and critical intake of zinc. Adaption, availability and interactions. Analyst 1995;120:913–5.
94. Sandstead HH. Requirements and toxicity of essential trace elements, illustrated by zinc and copper. Am J Clin Nutr 1995; 61(suppl):621S–4S.
95. Mulhem SA, Vessey AR, Taylor GL, Magruder LE. Suppression of antibody response by excess dietary zinc exposure during certain stages of ontogeny. Proc Soc Exp Biol Med 1985;180:453–61.
96. Beach RS, Gershwin ME, Hurley LS. Gestational zinc deprivation in mice: persistence of immunodeficiency for three generations. Science 1982;218:469–71.
97. Beach RS, Gershwin ME, Hurley LS. Reversibility of developmental retardation following murine fetal zinc deprivation. J Nutr 1982;112:1169–81.
98. Beach RS, Gershwin ME, Hurley LS. Persistent immunological consequences of gestational zinc deprivation. Am J Clin Nutr 1983;38:579–90.
99. Keller PR, Fraker PJ. Gestational zinc requirement of the A/J mouse: effects of a marginal zinc deficiency on in utero B cell development. Nutr Res 1986;6:41–50.
100. Vruwink KG, Hurley LS, Gershwin ME, Keen CL. Gestational zinc deficiency amplifies the regulation of metallothionein induction in adult mice. Proc Soc Exp Biol Med 1988;188:30–4.
101. Haynes DC, Gershwin ME, Golub MS, Cheung AT, Hurley LS, Hendrickx AG. Studies of marginal zinc deprivation in rhesus monkeys: VI. Influence on the immunohematology of infants in the first year. Am J Clin Nutr 1985;42:252–62.
102. Meadows NJ, Smith ME, Keeling PWN, et al. Zinc and small babies. Lancet 1981;2:1135–7.
103. Dutz W, Rossipal E, Ghavami H, Vessal K, Kohout E, Post C. Persistent cell mediated immune deficiency following infantile stress during the first six months of life. Eur J Pediatr 1976;122:117–30.
104. Ferguson AC. Prolonged impairment of cellular immunity in children with intrauterine growth retardation. J Pediatr 1978;93:52–6.
105. McFarlane H. Malnutrition and impaired immune response to infection. Proc Nutr Soc 1976;35:263–72.
106. Kearney JF, Bartles J, Hamilton AM, Lehuen A, Solvason J, Vakil M. Development and function of the early B cell repertoire. Int Rev Immunol 1992;8:247–57.
107. Vakil M, Kearney JF. Functional relationship between T15 and J558 idiotypes in BALB/c mice. Dev Immunol 1991;1:213–24.
108. Herzenberg LA, Kantor AB, Herzenberg L. Layered evolution in the immune system. A model for the ontogeny and development of multiple lymphocyte lineages. Ann N Y Acad Sci 1992;651:1–9.
109. Ward MM, Ward RE, Huang JH, Kohler H. Idiotope vaccine against *Streptococcus pneumoniae*. A precursor study. J Immunol 1987;139:2775–80.
110. Lucas AH, Granoff DM. Functional differences in idiotypically defined IgG1 anti-polysaccharide antibodies elicited by vaccination with *Haemophilus influenzae* type B polysaccharide-protein conjugates. J Immunol 1995;154:4195–202.
111. Seyoum G, Persaud TV. Influence of zinc on ethanol-induced placental changes in the rat. Histol Histopathol 1995;10:117–25.
112. Shrader RE, Hirsch KS, Levin J, Hurley LS. Attenuating effect of zinc on abnormal placental morphology in 6-mercaptopurine treated rats. Teratology 1978;17:315–25.
113. Keen CL, Gershwin ME. Zinc deficiency and immune function. Annu Rev Nutr 1990;10:415–31.
114. Tapazoglou E, Prasad AS, Hill G, Brewer GJ, Kaplan J. Decreased natural killer cell activity in zinc deficient subjects with sickle cell disease. J Lab Clin Med 1985;105:19–22.
115. Prasad AS, Kaplan J, Brewer GJ, Dardenne M. Immunological effects of zinc deficiency in sickle cell anemia (SCA). Prog Clin Biol Res 1989;319:629–49.
116. Abdallah JM, Kukuruga M, Nakeff A, Prasad AS. Cell cycle distribution defect in PHA-stimulated T lymphocytes of sickle cell disease patients. Am J Hematol 1988;28:279–81.
117. Bjorksten B, Black O, Gustavson KH, Hallmans G, Hagloff B, Tarvik A. Zinc and immune function in Down's syndrome. Acta Paediatr Scand 1980;69:183–7.
118. Licastro F, Chiricolo M, Mocchegiani E, et al. Oral zinc supplementation in Down's syndrome subjects decreased infection and normalized some humoral and cellular immune parameters. J Intellect Disabil Res 1994;38:149–62.
119. Labadie H, Verneau A, Trinchet JC, Beaugrand M. Does oral zinc improve the cellular immunity of patients with alcoholic cirrhosis? Gastroenterol Clin Biol 1986;10:799–803.
120. Cunningham-Rundles C, Cunningham-Rundles S, Garogalo J, et al. Increased T-lymphocyte function and thymopoietin following zinc repletion in man. Fed Proc 1979;38:1222 (abstr).
121. Sandstead HH. Understanding zinc: recent observations and interpretations. J Lab Clin Med 1994;124:322–7.
122. Prasad AS, Fitzgerald JT, Hess JW, Kaplan J, Pelen F, Dardenne M. Zinc deficiency in elderly patients. Nutrition 1993;9:218–24.
123. Garfinkel D. Is aging inevitable? The intracellular zinc deficiency hypothesis of aging. Med Hypotheses 1986;19:117–37.
124. Dardenne M, Boukaiba N, Gagnerault MC, et al. Restoration of the thymus in aging mice by in vivo zinc supplementation. Clin Immunol Immunopathol 1993;66:127–35.
125. Congy F, Clavel JR, le Devillechabrol A, Gerbet D, Marescot MR, Moulias R. Plasma zinc levels in elderly hospitalized subjects. Correlation with other nutritional and immunological markers and survival. Sem Hop 1983;59:3105–8.
126. Cossack ZT. T-lymphocyte dysfunction in the elderly associated with zinc deficiency and subnommal nucleoside phosphorylase activity: effect of zinc supplementation. Eur J Cancer Clin Oncol 1989;25:973–6.
127. Kaplan J, Hess JW, Prasad AS. Impaired interleukin 2 production in the elderly: association with mild zinc deficiency. J Trace Elem Exp Med 1988;1:3–8.
128. Payette H, Rola-Pleszczynski M, Ghadirian R. Nutrition factors in relation to cellular and regulatory immune variables in a free-living elderly population. Am J Clin Nutr 1990;52:927–32.
129. Duchateau J, Delepesse G, Vrijens R, Collet H. Beneficial effects of zinc supplementation on the immune response of old people. Am J Med 1981;70:1001–4.
130. Wagnet PA, Jemigan JJA, Bailey LB, Nickens C, Brazzi GA. Zinc nutriture and cell mediated immunity in the aged. Int J Vitam Nutr Res 1983;53:94–101.

131. Brummerstedt E, Flagstad T, Basse A, Anderson E. The effect of zinc on calves with hereditary thymic hypoplasia (lethal trait A 46). Acta Pathol Microbiol Scand 1971;79:686–7.
132. Brummerstedt E. Animal model of human disease. Acrodermatitis enteropathica, zinc malabsorption. Am J Pathol 1977;87:725–8.
133. Miller ER, Leucke RW, Ullrey DE, Baltzer BV, Bradley BL, Hoefer JA. Biochemical, skeletal and allometric changes due to zinc deficiency in the baby pig. J Nutr 1968;95:278–86.
134. Shanklin SH, Miller ER, Ullrey DE, Hoefer JA, Leucke RW. Zinc requirement of baby pigs on casein diets. J Nutr 1968;96:101–8.
135. Quartemman J. The effects of zinc deficiency or excess on the adrenals and the thymus in the rat. In: Hoekstra WG, Suttie JW, Gather HE, Mentz W, eds. Trace element metabolism in animals. Baltimore: University Park Press, 1974:742–8.
136. Haas SM, Fraker PJ, Luecke RW. The effect of zinc deficiency on the immune response of A/J mice. Fed Proc 1976;35:659 (abstr).
137. Julius R, Schilkind M, Sprinkle T, Rennert O. Acrodermatitis enteropathica with immune deficiency. J Pediatr 1973;83:1007–11.
138. Moynahan EJ. Acrodermatitis enteropathica: a lethal inherited human zinc-deficiency disorder. Lancet 1974;2:399–400.
139. Golden MHN, Jackson AA, Golden BE. Effect of zinc on thymus of recently malnourished children. Lancet 1977;2:1057–9.
140. Golden MHN, Golden BE, Harland PSEG, Jackson AA. Zinc and immunocompetence in protein-energy malnutrition. Lancet 1978;1:1226–8.
141. Beck FWJ, Kaplan J, Fine N, Handschu W, Prasad AS. Decreased expression of CD73 (ecto-5'-nulceotidase) in the CD8+ subset is associated with zinc deficiency in human patients. J Lab Clin Med 1997;130:147–56.
142. Pekarek RS, Sandstead HH, Jacob RA, Barcome DF. Abnormal cellular immune responses during acquired zinc deficiency. Am J Clin Nutr 1979;32:1466–71.
143. Allen JI, Kay NE, McClain CJ. Severe zinc deficiency in humans: association with a reversible T-lymphocyte dysfunction. Ann Intern Med 1981;95:154–7.
144. Sazawal S, Jalla S, Mazumder S, Sinha A, Black RE, Bhan MK. Effect of zinc supplementation on cell-mediated immunity and lymphocyte subsets in preschool children. Indian Pediatr 1997;34:589–97.
145. Kramer TR, Udomkesmalee E, Dhanamitta S, et al. Lymphocyte responsiveness of children supplemented with vitamin A and zinc. Am J Clin Nutr 1993;58:566–70.
146. Schlesinger L, Arevalo M, Arredondo S, Diaz M, Lönnerdal B, Stekel A. Effect of a zinc-fortified formula on immunocompetence and growth of malnourished infants. Am J Clin Nutr 1992;56:491–8.
147. Sempertegui F, Estrella B, Correa E, et al. Effects of short-term zinc supplementation on cellular immunity, respiratory symptoms, and growth of malnourished Equadorian children. Eur J Clin Nutr 1996;50:42–6.
148. Winchurch RA. Activation of thymocyte responses to interleukin-1 by zinc. Clin Immunol Immunopathol 1988;47:174–80.
149. Phillips JL, Azari P. Zinc transferrin. Enhancement of nucleic acid synthesis in phytohemagglutinin-stimulated human lymphocytes. Cell Immunol 1974;10:31–7.
150. Chvapil M. Effect of zinc on cells and biomembranes. Med Clin North Am 1976;60:799–812.
151. Malave I, Rodriguez J, Araujo Z, Rojas I. Effect of zinc on the proliferative response of human lymphocytes: mechanisms of its mitogenic action. Immunopharmacology 1990;20:1–10.
152. Tanaka Y, Shiozawa S, Morimoto I, Fujita T. Role of zinc in interleukin 2 (IL 2) mediated T-cell activation. Scand J Immunol 1990;31:547–52.
153. Ochi T, Sato K, Ohsawa M. Suppression of the proliferative response of human lymphocytes to cultured allogeneic HeLa cells by zinc. Toxicol Lett 1983;15:225–30.
154. Malave I, Rondon-Benaim I. Modulatory effect of zinc on the proliferative response of murine spleen cells to polyclonal T cell mitogens. Cell Immunol 1984;89:322–30.
155. Csermely R, Fodor R, Somogyi J. The tumor promoter tetradecanoylphorbol-13-acetate elicits the redistribution of heavy metals in subcellular fractions of rabbit thymocytes as measured by plasma emission spectroscopy. Carcinogenesis 1987;8:1663–6.
156. Driessen C, Hirv K, Kirchner H, Rink L. Zinc regulates cytokine induction by superantigens and lipopolysaccharide. Immunology 1995;84:272–7.
157. Fraser JD, Urban RG, Strominger JL, Robinson H. Zinc regulates the function of two superantigens. Proc Natl Acad Sci U S A 1992;89:5507–11.
158. McMahon LJ, Montgomery DW, Guschewsky A, Woods AH, Zukoski CF. In vitro effects of zinc chloride on spontaneous sheep red blood cell (E) rosette formation by lymphocytes from cancer patients and normal subjects. Immunol Commun 1976;15:53–67.
159. Martinotti S, Toniato E, Colagrande A, et al. Heavy-metal modulation of the human intercellular adhesion molecule (ICAM-1) gene expression. Biochim Biophys Acta 1995;1261:107–14.
160. Fraker PJ, King LE, Garvy BA, Medina CA. The immunopathology of zinc deficiency in humans and rodents: a possible role for programmed cell death. In: Klurfeld DM, ed. Human nutrition: a comprehensive treatise. New York: Plenum Press, 1993:267–83.
161. Fraker PJ, Osatiashtiani E, Wagner MA, King LE. Possible roles for glucocorticoids and apoptosis in the suppression of lymphopoiesis during zinc deficiency: a review. J Am Coll Nutr 1995;14:11–7.
162. Fraker PJ, Telford WG. A reappraisal of the role of zinc in life and death decisions of cells. Proc Soc Exp Biol Med 1997;215:229–36.
163. King LE, Fraker PJ. Flow cytometric analysis of the phenotypic distribution of splenic lymphocytes in zinc-deficient adult mice. J Nutr 1991;121:1433–8.
164. DePasquale-Jardieu P, Fraker PJ. Interference in the development of a secondary immune response in mice by zinc deprivation: persistence of effects. J Nutr 1984;114:1762–9.
165. Fraker PJ. Zinc deficiency: a common immunodeficiency state. Surv Immunol Res 1983;2:155–63.
166. Hart DA. Effect of zinc chloride on hamster lymphoid cells: mitogenicity and differential enhancement of lipopolysaccaride stimulation of lymphocytes. Infect Immun 1978;19:457–61.
167. Fraker PJ, Hildebrandt K, Luecke RW. Alteration of antibody-mediated responses of suckling mice to T-cell dependent and independent antigens by maternal marginal zinc deficiency: restoration of responsivity by nutritional repletion. J Nutr 1984;114:170–9.
168. Zazonico R, Fernandes G, Good RA. The differential sensitivity of T cell and B cell mitogenesis to in vitro Zn deficiency. Cell Immunol 1981;60:203–11.
169. Flynn A. Control of in vitro lymphocyte proliferation by copper, magnesium and zinc deficiency. J Nutr 1984;114:2034–42.
170. Fraker PJ, Jardieu P, Cook J. Zinc deficiency and immune function. Arch Dermatol Res 1987;123:1699–701.
171. Cunningham-Rundles S, Cunningham-Rundles C, Dupont B, Good RA. Zinc-induced activation of human B lymphocytes. Clin Immunol Immunopathol 1980;16:115–22.
172. Forbes IJ, Zalewski PD, Giannakis C. Role for zinc in a cellular response mediated by protein kinase C in human B lymphocytes. Exp Cell Res 1991;195:224–9.
173. Kirchner H, Salas M. Stimulation of lymphocytes with zinc ions. Methods Enzymol 1987;150:112–7.
174. Ferry E, Donner M. In vitro modulation of murine natural killer cytotoxicity by zinc. Scand J Immunol 1984;19:435–45.
175. Rajagopalan S, Winter CC, Wagtmann N, Long EO. The Ig-related killer cell inhibitory receptor binds zinc and requires zinc for recognition of HLA-C on target cells. J Immunol 1995;155:4143–6.
176. Borrego F, Lopez-Beltran A, Pena J, Solana R. Downregulation of Fc gamma receptor IIIA a (CD16-II) on natural killer cells induced by anti-CD16 mAb is independent of protein tyrosine kinases and protein kinase C. Cell Immunol 1994;158:208–17.
177. Sunzel B, Holm S, Reuterving CO, Soderberg T, Hallmans G, Hanstrom L. The effect of zinc on bacterial phagocytosis, killing

and cytoprotection in human polymorphonuclear leucocytes. APMIS 1995;103:635–44.
178. Wirth JJ, Fraker PJ, Kierszenbaum F. Zinc requirements for macrophage function: effect of zinc deficiency on uptake and killing of a protozoan parasite. Immunology 1989;68:114–9.
179. Wirth JJ, Fraker PJ, Kierszenbaum F. Changes in the level of marker expression by mononuclear phagocytes in zinc deficient mice. J Nutr 1984;114:1826–33.
180. Cook-Mills JM, Wirth JJ, Fraker PJ. Possible roles for zinc in destruction of *Trypansoma cruzi* by toxic oxygen metabolites produced by mononuclear phagocytes. Adv Exp Med Biol 1990;262:111–21.
181. James SJ, Swendseid M, Makinodan T. Macrophage-mediated depression of T-cell proliferation in zinc-deficient mice. J Nutr 1987;117:1982–8.
182. Ercan MT, Boor NM. Phagocytosis by macrophages in zinc-deficient rats. Int J Rad Appl Instrum 1991;18:765–8.
183. Humphrey PA, Ashraf M, Lee CM. Hepatic cells' mitotic and peritoneal macrophage phagocytic activities during *Trypanosoma musculi* infection in zinc-deficient mice. J Natl Med Assoc 1997; 89:259–67.
184. Karl L, Chvapil M, Zukoski CF. Effect of zinc on the viability and phagocytic capacity of peritoneal macrophages. Proc Soc Exp Biol Med 1973;142:1123–7.
185. Chvapil M, Stankova L, Bernard DS, Weldy RL, Carlson EVC, Campbell JB. Effect of zinc on peritoneal macrophages in vitro. Infect Immunol 1977;16:367–73.
186. Tone K, Suzuki T, Todoroki T. Influence of zinc deficiency on phagocytosis in mice. Kitasato Arch Exp Med 1991;64:263–9.
187. Supek F, Supekova L, Nelson N. Function of metal-ion homeostasis in the cell division cycle, mitochondrial protein processing, sensitivity to mycobacterial infection and brain function. J Exp Biol 1997;200:321–30.
188. Winchurch RA, Togo J, Adler WH. Supplemental zinc (Zn^{2+}) restores antibody formation in cultures of aged spleen cells. II. Effects on mediator production. Eur J Immunol 1987;17:127–32.
189. Scuderi R. Differential effects of copper and zinc on human peripheral blood monocyte cytokine secretion. Cell Immunol 1990; 126:127–32.
190. Driessen C, Hirv K, Rind L, Kirchner H. Induction of cytokines by zinc ions in human peripheral blood mononuclear cells and separated monocytes. Lymphokine Cytokine Res 1994;13:15–20.
191. Compton MM, Cidlowski JA. Thymocyte apoptosis. A model of programmed cell death. Trends Endocrinol Metab 1992;3:17–23.
192. Concordet JP, Ferry A. Physiological programmed cell death in thymocytes is induced by physical stress (exercise). Am J Physiol 1993;265:C626–9.
193. Zhang YH, Takahashi K, Jiang GZ, Kawai M, Fukada M, Yokochi T. In vivo induction of apoptosis (programmed cell death) in mouse thymus by administration of lipopolysaccharide. Infect Immun 1993;61:5044–8.
194. Quarterman J, Humphries WR. Effect of zinc deficiency and zinc supplementation on adrenals, plasma steroids and thymus in rats. Life Sci 1979;24:177–83.
195. DePasquale-Jardieu P, Fraker PJ. The role of corticosterone in the loss of immune function in the zinc deficient A/J mice. J Nutr 1979;109:1847–55.
196. DePasquale-Jardieu P, Fraker PJ. Further characterization of the role of corticosterone in the loss of humoral immunity in zinc deficient A/J mice as determined by adrenalectomy. J Immunol 1980;124:2650–5.
197. Garvy BA, King LE, Telford WG, Morford LA, Fraker PJ. Chronic elevation of plasma corticosterone causes reductions in the number of cycling cells of the B lineage in murine bone marrow and induces apoptosis. Immunology 1993;80:587–92.
198. Pleau JM, Fuentes V, Morgan JL, Bach JF. Specific receptor for the serum thymic factor (FTS) in lymphoblastoid cultured cell lines. Proc Natl Acad Sci U S A 1980;77:2861–5.
199. Cung MT, Marraud M, LeFrancier P, Dardenne M, Bach JF, Laussac JP. NMR study of a lymphocyte differentiating thymic factor. An investigation of the Zn (II)-nonapeptide complexes (thymulin). J Biol Chem 1988;263:5574–80.
200. Dardenne M, Pleau JM, Nabbara B, et al. Contribution of zinc and other metals to the biological activity of the serum thymic factor. Proc Natl Acad Sci U S A 1982;79:5370–3.
201. Iwata T, Incefy GS, Tanaka T, et al. Circulating thymic hormone levels in zinc deficiency. Cell Immunol 1979;47:100–5.
202. Iwata T, Incefy GS, Cunningham-Rundles S, et al. Circulating thymic hormone activity in patients with primary and secondary immunodeficiency diseases. Am J Med 1981;71:385–94.
203. Bendtzen K. Differential role of Zn^{2+} in antigen-and mitogen-induced lymphokine production. Scand J Immunol 1980;12:489–92.
204. Flynn A, Loftus MA, Finke JH. Production of interleukin-1 and interleukin-2 in allogeneic mixed lymphocyte cultures under copper, magnesium and zinc deficient conditions. Nutr Res 1984; 4:673–9.
205. Smit V, van Veelen PA, Tjaden UR, van der Greef J, Haaijman JJ. Human interleukin-3 contains a discontinuous zinc-binding domain. Biochem Biophys Res Commun 1992;187:859–66.
206. Radhakrishnan R, Walter LJ, Hruza A, et al. Zinc mediated dimer of human interferon-alpha 2b revealed by X-ray crystallography. Structure 1996;4:1453–63.
207. Salvin SB, Rabin BS. Resistance and susceptibility to infection in inbred murine strains. IV. Effects of dietary zinc. Cell Immunol 1984;87:546–52.
208. Romagnani S. The Th1/Th2 paradigm. Immunol Today 1997; 18:263–6.
209. Beck FWJ, Prasad AS, Kaplan J, Fitzgerald JT, Brewer GJ. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. Am J Physiol 1997; 272:E1002–7.
210. Prasad AS, Beck FWJ, Grabowski SM, Kaplan J, Mathog RH. Zinc deficiency: changes in cytokine production and T-cell subpopulations in patients with head and neck cancer in non-cancer subjects. Proc Assoc Am Physicians 1997;109:68–77.
211. Cheek DB, Hay HJ, Lattanzio L, Ness D, Ludwigsen N, Spargo R. Zinc content of red and white blood cells in aboriginal children. Aust N Z J Med 1984;14:638–42.
212. Blazsek I, Mathe G. Zinc and immunity. Biomed Pharmacother 1984;38:187–93.
213. Cousins RJ. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol Rev 1985;65:238–309.
214. Ackland ML, McArdle HJ. Significance of extracellular zinc-binding ligands in the uptake of zinc by human fibroblasts. J Cell Physiol 1990;145:409–13.
215. Kalfakakou V, Simons TJB. Anionic mechanisms of zinc uptake across the human red cell membrane. J Physiol 1990;421:485–97.
216. Palmiter RD, Cole TB, Findley SD. ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. EMBO J 1996;15:1784–891.
217. Palmiter RD, Findley SD. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. EMBO J 1995;14:639–49.
218. Cousins RJ, Lee-Ambrose LM. Nuclear zinc uptake and interactions and metallothionein gene expression are influenced by dietary zinc in rats. J Nutr 1992;122:56–64.
219. Chesters JK. Biochemistry of zinc in cell division and tissue growth. In: Mills CR, ed. Zinc in human biology. New York: Springer-Verlag, 1989:109–18.
220. Grummt F, Weimann-Dorsch C, Schneider-Schaulies J, Lux A. Zinc as a second messenger of mitogenic induction. Effects on diadenosine tetraphosphate (Ap4A) and DNA synthesis. Exp Cell Res 1986;163:191–200.
221. Chausmer AB, Chausmer AL, Dajani N. Effect of mitogenic and hormonal stimulation on zinc transport in mixed lymphocyte cultures. J Am Coll Nutr 1991;10:205–8.
222. Prasad AS, Beck FW, Endre L, Handschu W, Kukuruga M, Kumar

210

G. Zinc deficiency affects cell cycle and deoxythymidine kinase gene expression in HUT-78 cells. J Lab Clin Med 1996;128:9–11.
223. Chesters JK, Petrie L, Lipson HE. Two zinc-dependent steps during G1 to S phase transition. J Cell Physiol 1993;155:445–51.
224. Falchuk KH, Krishan A. 1,10-Phenanthroline inhibition of lymphoblast cell cycle. Cancer Res 1977;37:2050–6.
225. Serrano L, Dominguez JE, Avila J. Identification of zinc binding sites of proteins: zinc binds to the amino terminal region of tubulin. Anal Biochem 1988;172:210–8.
226. Hesketh JE. Impaired microtubule assembly in brain from zinc-deficient pigs and rats. Int J Biochem 1981;13:921–6.
227. Slater JR, Mildvan AS, Loeb LA. Zinc in DNA polymerizes. Biochem Biophys Res Commun 1971;44:37–43.
228. Falchuk KH, Krishan A, Vallee BL. DNA distribution in cell-cycle of Euglena-Gracilis cytofluorometry of zinc-deficient cells. Biochemistry 1975;14:3439–44.
229. Kirchgessner M, Roth HP, Weigand E. Biochemical changes in zinc deficiency in trace elements. In: Prasad AS, Oberleas D, eds. Human health and disease. New York: Academy Press, 1976: 189–225.
230. Brudzynska K, Ploszajska A, Roszczyk R, Walter Z. Interaction of zinc ions with DNA-dependent RNA polymerase-A,B,C isolated from calf thymus. Mol Biol Rep 1982;8:77–83.
231. Good RA, West A, Day NK, Dong ZW, Fernandes G. Effect of undernutrition on host cell and organ function. Cancer Res 1982;42:737–46.
232. Williams RJP. Zinc: what is its role in biology? Endeavour 1984;8:65–70.
233. Coleman JE. Zinc proteins: enzymes, storage proteins, transcription factors and replication proteins. Annu Rev Biochem 1992;61:897–946.
234. Morimoto S, Shiraishi T, Fukuo K, et al. EDTA induces differentiation and suppresses proliferation of promyelocytic leukemia cell line HL60-possible participation of zinc. Biochem Int 1992; 28:313–21.
235. Flamigni E, Campana G, Carboni L, Guarnieri C, Spampinato S. Zinc is required for the expression of ornithine decarboxylase in a difluoromethylornithine-resistant cell line. Biochem J 1994; 299:515–9.
236. Wellinghausen N, Kirchner H, Rink L. The immunobiology of zinc. Immunol Today 1997;18:519–21.
237. Palmiter RD. Regulation of metallothionein genes by heavy metals appears to be mediated by a zinc-sensitive inhibitor that interacts with a constitutively active transcription factor, MTF-1. Proc Natl Acad Sci U S A 1994;91:1219–23.
238. Saurin AJ, Borden KL, Boddy MN, Freemont PS. Does this have a familiar RING? Trends Biochem Sci 1996;21:208–14.
239. Csermely P, Somogyi J. Zinc as a possible mediator of signal transduction in T lymphocytes. Acta Physiol Hung 1989;74:195–9.
240. Turner JM, Brodsky MH, Irving BA, Levin SD, Perlmutter RM, Littman DR. Interaction of the unique N-terminal region of tyrosine kinase p56lck with cytoplasmic domains of CD4 and CD8 is mediated by cysteine motifs. Cell 1990;60:755–65.
241. Pemelle JJ, Creuzet C, Loeb J, Gacon G. Phosphorylation of the lymphoid cell kinase p56lck is stimulated by micromolar concentrations of Zn^{2+}. FEBS Lett 1991;281:278–82.
242. Moser C, Roth HP, Kirchgessner M. Influence of alimentary zinc deficiency on the concentration of the second messengers D-myo-inositol-1,4,5-triphosphate (IP3) s.n.-1,2-diacylglycerol (DAG) in testes and brain of force-fed rats. Biol Trace Elem Res 1996; 52:281–91.
243. Zalewski PD, Forbes IJ. Intracellular zinc and the regulation of apoptosis. In: Lavin M, Watters D, eds. Programmed cell death: the cellular and molecular biology of apoptosis. Melbourne: Harwood Academic Publishers, 1993:73–86.
244. Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 1980;284:555–6.
245. Cohen JJ, Duke RC, Fadok VA, Sellins KS. Apoptosis and programmed cell death in immunity. Annu Rev Immunol 1992; 10:267–93.
246. Dinsdale D, Williams RB. The enhancement by dietary zinc deficiency of the susceptibility of the rat duodenum to colchicine. Br J Nutr 1977;37:135–42.
247. Elmes ME. Apoptosis in the small intestine of zinc-deficient and fasted rats. J Pathol 1977;123:219–24.
248. Elmes ME, Jones JG. Ultrastructural changes in the small intestine of zinc-deficient rats. J Pathol 1980;130:37–43.
249. Elmes ME, Jones JG. Ultrastructural studies on Paneth cell apoptosis in zinc deficient rats. Cell Tissue Res 1980;208:57–63.
250. Waring P, Egan M, Braithwaite A, Mullbacher A, Sjearda A. Apoptosis induced in macrophages and T blasts by the mycotoxin sporidesmin and protection by Zn^{2+} salts. Int J Immunopharmacol 1990;12:445–57.
251. Thomas DJ, Caffrey TC. Lipopolysaccharide induces doublestranded DNA fragmentation in mouse thymus: protective effect of zinc pretreatment. Toxicology 1991;68:327–37.
252. Martin SJ, Mazdai G, Strain JJ, Cotter TG, Hannigan BM. Programmed cell death (apoptosis) in lymphoid and myeloid cell lines during zinc deficiency. Clin Exp Immunol 1991;83:338–43.
253. Zalewski PD, Forbes IJ, Giannakis C. Physiological role for zinc in prevention of apoptosis (gene-directed death). Biochem Int 1991;24:1093–101.
254. McCabe MI, Jiang SA, Orrenius S. Chelation of intracellular zinc triggers apoptosis in mature thymocytes. Lab Invest 1993;69:101–9.
255. Treves S, Trentini PL, Ascanelli M, Bucci G, Divirgilio F. Apoptosis is dependent on intracellular zinc and independent of intracellular calcium in lymphocytes. Exp Cell Res 1994;211:339–43.
256. Zalewski PD, Forbes IJ, Betts WH. Correlation of apoptosis with change in intracellular labile Zn, using Zinquin, a new specific fluorescent probe for Zn. Biochem J 1993;296:403–8.
257. Giannakis C, Forbes IJ, Zalewski PD. Ca^{2+}/Mg^{2+}-dependent nuclease: tissue distribution, relationship to inter-nucleosomal DNA fragmentation and inhibition by Zn^{2+}. Biochem Biophys Res Comm 1991;181:915–20.
258. Lohmann RD, Beyersmann D. Cadmium and zinc mediated changes of the Ca(2+)-dependent endonuclease in apoptosis. Biochem Biophys Res Comm 1993;190:1097–103.
259. Ballester OF, Prasad AS. Anergy, zinc deficiency, and decreased nucleoside phosphorylase activity in patients with sickle cell anemia. Ann Intern Med 1983;98:180–2.
260. Meflah S, Prasad AS. Nucleotides in lymphocytes from human subjects with zinc deficiency. J Lab Clin Med 1989;114:114–9.
261. Seres DS, Bunk MJ, Osborne MP, Rivlin RS, Tiwari RK. Effects of marginal dietary zinc deficiency and vitamin E supplementation on hepatic adenine dinucleotide phosphoribosyl transferase activity in female Sprague Dawley rats. Nutr Res 1991;11:337–45.
262. Rice WG, Schaeffer CA, Hanan B, et al. Inhibition of HIV-1 infectivity by zinc-ejecting aromatic C-nitroso compounds. Nature 1993;361:473–5.
263. Zalewski PD, Forbes IJ, Seamark RF, et al. Flux of intracellular labile zinc during apoptosis (gene-directed cell death) by a specific chemical probe, Zinquin. Chem Biol 1994;1:153–61.
264. Chvapil M, Elias SL, Ryan JN, Zukoski CF. Pathophysiology of zinc. In: International review of neurobiology. Supplement 1st ed. New York: Academy Press, 1972:105–24.
265. Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. Free Radic Biol Med 1990;8:281–91.
266. Alexander I, Forre O, Aaseth J, Dobloug J, Ovrebo S. Induction of a metallothionein-like protein in human lymphocytes. Scand J Immunol 1982; 15:217–20.
267. Chvapil M. New aspects in the biological role of zinc: a stabilizer of macromolecules and biological membranes. Life Sci 1973; 13:1041–9.
268. Bettger WJ, O'Dell BL. A critical physiological role of zinc in the structure and function of biomembranes. Life Sci 1981; 28:1425–38.
269. Kroncke KD, Fehsel K, Schmidt T, et al. Nitric oxide destroys zinc-sulfer clusters inducing zinc release from metallothionein and inhibition of the zinc finger-type yeast transcription activator LAC9. Biochem Biophys Res Commun 1994;200:1105–10.

211

270. Taylor CG, Bray TM. Effect of hyperoxia on oxygen free radical defense enzymes in the lung of zinc-deficient rats. J Nutr 1991; 121:460–6.

271. Caulfield LE, Zavaleta N, Shankar AH, Merialdi M. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. Am J Clin Nutr 1998;68:499S–508S.

OTHER

Implications of the Institute of Medicine Weight Gain Recommendations for Preventing Adverse Pregnancy Outcomes in Black and White Women

Laura E. Caulfield, PhD, Rebecca J. Stoltzfus, PhD, and Frank R. Witter, MD

ABSTRACT

Objectives. This study examined the relation between gestational weight gain and risk of delivering a small-for-gestational-age or large-for-gestational-age infant by race, along with the implications of gaining weight according to the Institute of Medicine guidelines.

Methods. Logistic regression methods were used to identify risk factors for small- and large-for-gestational-age births among 2617 Black and 1253 White women delivering at the Johns Hopkins Hospital between 1987 and 1989.

Results. Rate of total weight gain was related to risk of small- and large-for-gestational-age births; the relationship differed according to maternal body mass index but not race. No differences in outcome by race were evident for women with low body mass indexes; among those with average or high indexes, however, Black women were at higher risk of small-for-gestational-age births and at lower risk of large-for-gestational-age births.

Conclusions. Having Black women gain at the upper end of the recommended range is unlikely to produce measurable reductions in small-for-gestational-age births. Some beneficial reductions in the risk of large-for-gestational-age births may occur if weight gain recommendations are lowered for average-weight and overweight White women. (*Am J Public Health.* 1998;88:1168–1174)

Introduction

In 1990, the Institute of Medicine published guidelines for gestational weight gain based on a woman's prepregnancy body mass index (weight in kilograms divided by height in meters squared).[1] Women entering pregnancy with a body mass index of less than 19.8 kg/m^2 should gain 12.7 to 18.2 kg, those with an index between 19.8 and 26.0 kg/m^2 should gain 11.4 to 15.9 kg, and those with an index between 26.1 and 29 kg/m^2 should gain 6.8 to 11.4 kg. For women with a body mass index of more than 29.0 kg/m^2, only a lower threshold of 6.0 kg was specified. It was also recommended that adolescents and Black women gain at the upper end of the recommended ranges.

The Institute of Medicine urged researchers to investigate the usefulness of the weight gain recommendations for preventing adverse pregnancy outcomes across populations and within specific ethnic groups.[1] Since then, studies have evaluated the institute's recommendations for decreasing the risk of low as well as high birthweight and cesarean delivery.[2–6] Questions remain, however, regarding the benefits of race-specific weight gain recommendations.

We have shown previously that only one third of women delivering at Johns Hopkins Hospital gain the recommended amounts of weight during pregnancy, with Black women more likely to undergain and White women more likely to overgain.[7] To understand the implications of these findings, we examined the relation between weight gain and pregnancy outcome among Black and White women delivering at that hospital. We addressed 4 questions: (1) Does weight gain influence the risk of delivering a small-for-gestational-age or large-for-gestational-age infant? (2) Do risks differ by prepregnancy body mass index or race? (3) Should Black women be advised to gain at the upper ends of the recommended weight gain ranges? and (4) What would be the impact on the incidences of small- and large-for-gestational-age births if women gained the recommended amounts of weight, and how would this compare with other interventions such as smoking prevention or optimizing prepregnancy body mass index?

Methods

The Johns Hopkins Hospital obstetric database contains information on deliveries of all live-born infants at the hospital between 1987 and 1989. The data were abstracted from clinical records at discharge by trained personnel. For analyses, we identified all Black or White women giving birth over the 3-year period who had singleton pregnancies of at least 28 weeks' duration and who provided information on prepregnancy weight, height, and total gestational weight gain. Of 6566 pregnancies, 2421 were excluded as a result of missing values for these variables, 98 were excluded owing to improbable information, and 24 were excluded owing to non-Black/non-White ethnicity. A computer program randomly chose one delivery per woman to remain in the sample, resulting in a final sample of 3870 independent deliveries.

Analyses comparing births included in the sample with those excluded showed

Laura E. Caulfield and Rebecca J. Stoltzfus are with the Center for Human Nutrition, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md. Frank R. Witter is with the Department of Gynecology and Obstetrics, Division of Maternal-Fetal Medicine, Johns Hopkins School of Medicine.

Requests for reprints should be sent to Laura E. Caulfield, PhD, Center for Human Nutrition, Johns Hopkins University School of Hygiene and Public Health, Room 2041, 615 N Wolfe St, Baltimore, MD 21205 (e-mail: lcaulfie@jhsph.edu).

This paper was accepted February 17, 1998.

Reprint

214

many statistical differences resulting from the large sample size but only a few differences of practical significance. There were no differences in maternal race. However, excluded women were more likely to be multiparous service patients with little or no prenatal care, and they were twice as likely to deliver a small- or large-for-gestational-age infant. Thus, the analytic sample was at somewhat lower risk than the general Johns Hopkins Hospital obstetric population.

Maternal height and prepregnancy weight were self-reported. Women were classified into categories according to their body mass index: less than 19.8 kg/m^2 (thin), 19.8 to 26.0 kg/m^2 (average), 26.1 to 29.0 kg/m^2 (overweight), or more than 29.0 kg/m^2 (very overweight).[1] Because few overweight women delivered small-for-gestational-age infants (n = 19), overweight and very overweight women were combined for analyses. This was reasonable because the 2 groups had similar recommended weight gains,[5,6] and preliminary analyses indicated that the groups were comparable in terms of weight gain[7] and the relation between weight gain and pregnancy outcome. Women were weighed at each visit, and total weight gain was calculated as the difference between prepregnancy weight and final recorded weight before delivery. For analyses, we used rate of weight gain, calculated as total weight gain (in grams) divided by duration of pregnancy (date of delivery minus date of last menses).

Birthweights were compared with a reference distribution of birthweights by week of gestation.[8] Infants considered small for their gestational age had birthweights at less than the 10th percentile for their week of gestation and sex. Infants considered large for their gestational age had birthweights above the 90th percentile for their week of gestation; girls and boys were not considered separately, because sex-specific values were not available.[8] Infants with birthweights between the 10th and 90th percentiles were considered to be of adequate weight for gestation. Examining outcomes in terms of fetal growth adequacy as it related to rate of weight gain adjusted up front for duration of pregnancy. Duration of pregnancy did not remain in the final regression models, thus validating the use of this adjustment strategy.

Other variables considered during analyses included maternal race, age, parity, years of schooling, smoking, provider type, diabetes, hypertension, and the sex of the fetus. Maternal race was self-reported. Smoking (yes/no) was defined on the basis of maternal reporting of at least one cigarette per day. For diabetes and hypertension, preexisting and gestation-related cases were combined. Provider types were managed care, service, and private.

Characteristics of women delivering small- or large-for-gestational-age infants were compared with those of women delivering adequate-weight-for-gestational-age infants via t tests and χ^2 tests. Analyses were stratified by prepregnancy body mass index and race. Distributions of continuous variables were also categorized into quantiles and recompared across categories of pregnancy outcome. Exploratory analyses indicated, for example, that maternal age should be kept as a continuous variable and that it was reasonable to treat smoking as a dichotomous variable. Furthermore, over the range of observed weight gains, the effect of increasing weight gain on the frequency of each pregnancy outcome within body mass index strata was constant or incremental and did not differ in magnitude or statistical significance by race.

Logistic regression models were developed to identify determinants of delivering a small- or large-for-gestational-age infant, as opposed to an adequate-weight-for-gestational-age infant, within each prepregnancy body mass index stratum. Because of the body mass index range within each stratum, body mass index was also included as a continuous variable. Full models were fitted, and these models included most known risk factors for small- and large-for-gestational-age births.[1] Factors were retained in each model with $P < .05$ as the criterion for significance; for consistency, however, risk factors significant in one model were retained in the other 2 models. Interaction terms, including product terms for each variable with race and weight gain, were tested for inclusion in the models ($P < .15$). Few interaction terms were identified, and, because their contribution to the overall χ^2 value was marginal and had little influence on the interpretation of the findings, interactions were dropped from the final models. Odds ratios and their 95% confidence intervals (CIs) were calculated from the 6 final models.

To describe the impact of variation in weight gain on the probability of delivering a small- or large-for-gestational-age infant, we graphed the probability of each outcome associated with weight gain in each of the models. Each outcome probability was the exponentiation of the logistic model formula, which was linear in the parameter estimates (multiplied by given values of the set of maternal or other characteristics influencing the likelihood of the outcome and divided by one plus the exponentiation of this quantity). We focused on predicted probability because the line's placement illustrated the overall probability of the outcome and the slope represented the expected impact of unit changes in weight gain. Differences in placement of the lines across strata also illustrated the effect of varying prepregnancy body mass index values. For presentation, we converted rate of weight gain to expected total weight gain at term by multiplying each rate by the average pregnancy duration of the sample (38.5 weeks).

We calculated the proportion of small- and large-for-gestational-age infants attributable to each of several modifiable risk factors[9] and estimated the expected impact on the incidences of such births if women gained weight within the recommended ranges for their body mass index. We also considered the impact of eradicating maternal smoking during pregnancy and decreasing the proportion of women entering pregnancy with body mass indexes of less than 19.8 kg/m^2 or greater than 26.0 kg/m^2. Estimates were made for each race and body mass index stratum separately. These estimates were then summed to allow consideration of differences in exposure by race and body mass index strata and to illustrate the differential impact on incidence by race associated with modifying prepregnancy body mass indexes.

Results

Within each body mass index stratum, Black women were younger and more likely to be service patients, to smoke during pregnancy, and to gain less total weight than White women (Table 1). Also, Black women were about twice as likely to deliver a small-for-gestational-age infant and one third as likely to deliver a large-for-gestational-age infant.

The unadjusted change in the relative odds of small- or large-for-gestational-age births associated with weight gain was constant within each body mass index stratum but varied across strata. Among thin women, each additional 50 g per week of weight gain (2.2 kg over the pregnancy) diminished the risk of a small-for-gestational-age birth by 18% (95% CI = 6%, 31%), whereas among overweight women, each additional 50 g per week diminished this risk only by 5% (95% CI = 0%, 14%). The risk of large-for-gestational-age births increased by 22% (10% to 34%) for each additional 50 g per week among thin women but only by 12% (6% to 17%) among overweight women. Across all strata, the effect of weight gain on pregnancy outcomes was not significantly different for Black and White women ($P > .15$).

Adjusted risks of delivering a small- or large-for-gestational-age infant (vs an infant

TABLE 1—Characteristics of 3870 Women Delivering at Johns Hopkins Hospital, According to Prepregnancy Body Mass Index (BMI) and Race

	BMI < 19.8 kg/m²		BMI 19.8–26.0 kg/m²		BMI > 26.0 kg/m²	
Characteristic	Black	White	Black	White	Black	White
No.	523	267	1479	796	615	190
Age, y, mean ± SD	21.7 ± 4.8	27.1 ± 6.6	22.7 ± 5.3	29.8 ± 5.8	24.9 ± 6.0	28.2 ± 5.5
Primiparous, %	52.4	55.4	50.1	48.0	36.9	46.9
Provider type, %						
Private	4.6	62.3	6.3	72.8	8.0	59.7
Managed care	8.3	9.7	11.4	6.0	10.8	10.5
Service	87.1	28.0	82.3	21.2	81.2	29.8
Height (in)	64.3 ± 2.8	65.3 ± 2.6	63.6 ± 2.7	64.5 ± 2.7	63.7 ± 2.7	64.6 ± 3.0
BMI, kg/m², mean ± SD	18.4 ± 1.0	18.5 ± 1.0	22.7 ± 1.8	22.1 ± 1.8	31.2 ± 4.8	31.2 ± 5.2
Total weight gain, kg, mean ± SD	13.3 ± 5.7	14.6 ± 5.1	13.6 ± 6.7	15.3 ± 5.4	12.4 ± 7.7	14.5 ± 7.3
Rate of weight gain, g/wk, mean ± SD	345 ± 142	376 ± 123	351 ± 171	395 ± 136	319 ± 198	372 ± 187
Smoking, %	32.8	20.6	35.4	20.0	28.8	25.4
Hypertension, %	4.3	3.0	6.0	5.7	11.9	17.0
Duration of pregnancy, wk, mean ± SD	38.2 ± 2.3	38.7 ± 2.4	38.4 ± 2.3	38.8 ± 2.2	38.8 ± 2.3	38.9 ± 2.3
Fetal growth, %						
SGA	9.9	5.6	6.6	3.1	7.0	5.3
AGA	86.9	85.0	89.2	83.8	85.2	65.2
LGA	3.2	9.4	4.2	13.1	7.8	29.5

Note. SGA = small for gestational age; AGA = adequate for gestational age; LGA = large for gestational age.

of adequate weight for gestational age) were calculated within each body mass index stratum (Table 2). After adjustment, increasing rates of weight gain still reduced the risk of small-for-gestational-age births and increased the risk of large-for-gestational-age births within each stratum. Maternal race no longer influenced the risk of either type of birth among thin women after adjustment, but it still differentiated risk among women with average and high body mass indexes.

Figure 1 shows the predicted probabilities of delivering small- and large-for-gestational-age infants associated with expected total weight gain at term for Black and White women in each body mass index stratum. First, within each stratum, increasing weight gain was associated with a decreased risk of delivering a small-for-gestational-age infant and an increased risk of delivering a large-for-gestational-age infant. For example, among thin women, the risk of delivering a small-for-gestational-age infant declined from 8.3% to 3.3% among Black women and from 7.7% to 3.0% among White women over the range of weight gain, while the risk of delivering a large-for-gestational-age infant increased from 1.0% to 4.7% among Black women and from 1.4% to 6.6% among White women. Second, the effect of weight gain varied by maternal body mass index. In comparison with the risk of small-for-gestational-age births for White women with low body mass indexes (described earlier), risks declined from 3.7% to 1.6% for White women with average body mass indexes and from 5.6% to 2.9% for White women with high indexes. Third, there were no differences in incidence by race for women with low body mass indexes. However, at higher body mass indexes, racial disparities in risk of small-for-gestational-age births increased, and more so for large-for-gestational-age births.

Effects of prepregnancy body mass index on pregnancy outcomes can be seen by examining how the predicted probabilities of small- and large-for-gestational-age births vary across the 3 graphs (Figure 1). Among White women, a body mass index of 19.8 to 26.0 kg/m² (vs an index of less than 19.8 kg/m²) was associated with a lower overall risk of delivering a small-for-gestational-age infant (2.7% vs 5.4% for a total weight gain of 12.5 kg), but an index of greater than 26.0 kg/m² did not diminish further the risk of such a birth (4.2% for a weight gain of 12.5 kg). Among Black women, the incidence of small-for-gestational-age births remained stable, changing from 5.9% to 5.4% and 6.0% as body mass index increased. The risk of large-for-gestational-age births was influenced more by prepregnancy body mass index. Again, for White women, the change in risk was greater, with the incidence (for a 12.5-kg gain) increasing from 2.6% to 6.8% to 12.0% across body mass index strata; for Black women, the incidence increased from 1.8% to 3.6% to 6.4%.

Predicted changes in the incidences of small- and large-for-gestational-age births associated with changes in weight gain were calculated and compared with the expected changes in incidence associated with optimizing prepregnancy body mass index or eradicating maternal smoking (Table 3). Trade-offs exist for preventing small- and large-for-gestational-age births associated with changes in weight gain, body mass index, and smoking. Reducing the proportion of women gaining less weight than recommended (undergain) would probably result in an 11% to 16% reduction in small-for-gestational-age births but, concomitantly, a 17% to 26% increase in large-for-gestational-age births. Conversely, reducing the proportion of women gaining more weight than recommended (overgain) would decrease the latter but increase the former. Having Black women enter pregnancy with average body mass indexes would have a minimal impact on small-for-gestational-age births. Preventing undergain and smoking would have the greatest impact on reducing such births among Black women, whereas preventing low body mass index and smoking would have the greatest impact on reducing these births among White women. Reductions in large-for-gestational-age births could be achieved by preventing high body mass indexes and overgain during pregnancy.

Discussion

Rate of gestational weight gain was related in a constant fashion to the likelihood of delivering a small- or large-for-gestational-age infant as compared with an adequate-weight-for-gestational-age infant. The influ-

216

TABLE 2—Adjusted Odds Ratios for Small- or Large-for-Gestational-Age Births (vs Adequate-Weight-for-Gestational-Age Births), According to Maternal Prepregnancy Body Mass Index (BMI)

	Odds Ratio (95% Confidence Interval)					
	Small for Gestational Age			Large for Gestational Age		
Characteristic	BMI < 19.8	BMI 19.8–26.0	BMI > 26.0	BMI < 19.8	BMI 19.8–26.0	BMI > 26.0
Black race	1.09 (0.58, 2.06)	2.03 (1.19, 3.48)	1.46 (0.61, 3.46)	0.70 (0.28, 1.72)	0.51 (0.32, 0.83)	0.50 (0.29, 0.86)
Multiparity	0.65 (0.36, 1.18)	0.53 (0.35, 0.80)	0.53 (0.27, 1.02)	2.54 (1.26, 5.13)	1.62 (1.15, 2.26)	1.59 (1.00, 2.54)
Any smoking	2.32 (1.34, 4.00)	3.40 (2.31, 5.02)	2.33 (1.27, 4.29)	0.74 (0.30, 1.87)	0.68 (0.44, 1.06)	0.55 (0.31, 0.96)
Height (per 2.8 in)	0.68 (0.51, 0.89)	0.73 (0.59, 0.89)	0.80 (0.59, 1.09)	1.95 (1.34, 2.86)	1.40 (1.17, 1.68)	1.12 (0.90, 1.39)
Body mass index (per kg/m^2)	0.83 (0.65, 1.07)	1.00 (0.90, 1.11)	0.98 (0.92, 1.06)	1.27 (0.87, 1.84)	1.12 (1.02, 1.22)	1.09 (1.04, 1.13)
Rate of weight gain (per 50 g/wk)	0.87 (0.78, 0.97)	0.90 (0.84, 0.96)	0.93 (0.86 1.01)	1.25 (1.11, 1.41)	1.14 (1.08, 1.20)	1.13 (1.07, 1.20)
Age (per 6 years)	0.95 (0.51, 1.76)	1.37 (1.11, 1.70)	0.86 (0.61, 1.22)	...	...	...
Provider type						
Managed care[a]	0.86 (0.32, 2.28)	2.44 (1.48, 4.03)	0.44 (0.13, 1.45)	...	...	...
Private[a]	...	...	...	2.55 (0.99, 6.54)	2.25 (1.39, 3.63)	1.69 (0.95, 3.01)
Hypertension	1.91 (0.62, 5.94)	1.93 (0.97, 3.81)	2.22 (1.06, 4.66)	...	...	...
Female infant	...	...	...	0.57 (0.28, 1.17)	1.43 (1.02, 1.99)	0.74 (0.47, 1.15)

Note. Presented are the adjusted odds ratios for each variable in each of 6 final models (2 outcomes × 3 prepregnancy BMI categories).
[a]As compared with all other provider types.

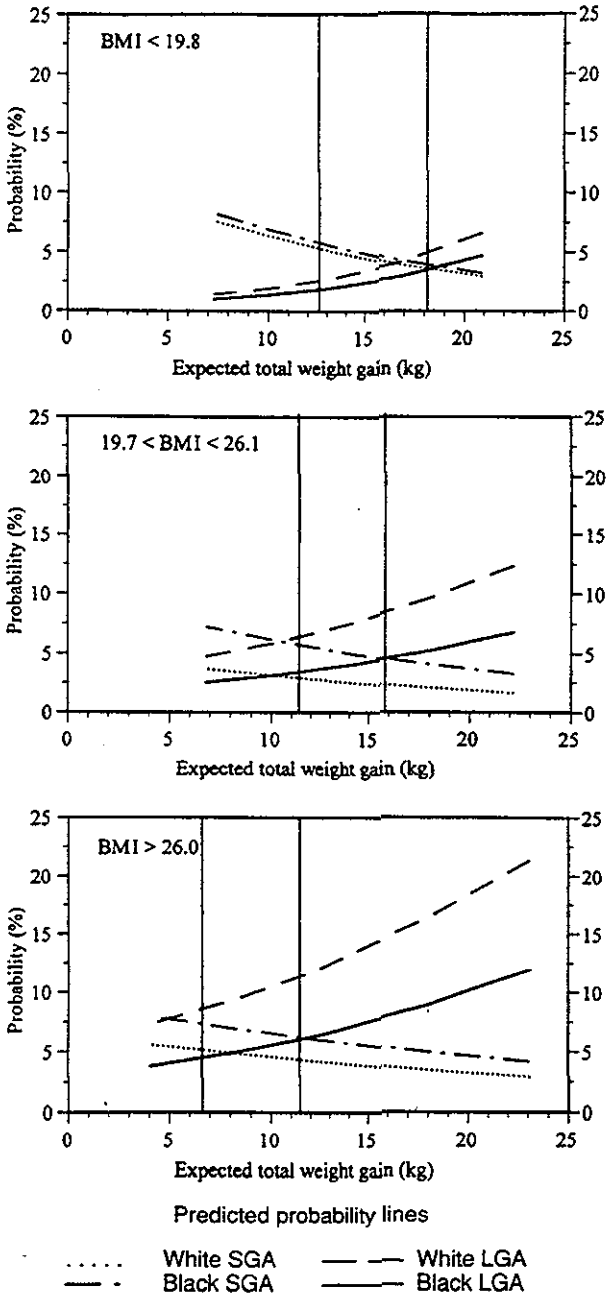
ence of weight gain on the risk of small- and large-for-gestational-age births was modified by maternal body mass index, confirming the findings of the Institute of Medicine and other investigators.[1,2,4,5] The relation between weight gain and risk of small- or large-for-gestational-age births did not differ by maternal race in any of the body mass index strata, but the effect of maternal race on pregnancy outcome did differ across these strata. After adjustment for confounding factors, maternal race no longer influenced risk among thin women; among those with body mass indexes greater than 19.8 kg/m^2, however, Black women were still more likely to deliver a small-for-gestational-age infant and less likely to deliver a large-for-gestational-age infant.

Before the implications of these findings are considered, several limitations of the data should be addressed. First, we relied on self-reported prepregnancy weight. Previous research indicates excellent agreement between actual and self-reported heights and weights, with greater underreporting of weight at higher body mass index levels but no difference in agreement by race.[10–12] Thus, errors in body mass index probably diminish the effect of body mass index on pregnancy outcomes—particularly for heavier women—but would not differentially affect results by race. Underreporting of higher prepregnancy weights may also explain the observed similarities between overweight and very overweight women and provide further justification for our grouping these women together. Second, despite these considerations, our inability to analyze outcomes for overweight and very overweight women separately is a limitation. Third, we calculated weight gain using reported prepregnancy weight and last weight recorded before delivery. At Johns Hopkins Hospital, women delivering at term were probably weighed within 1 week of delivery; thus, for most women, gestational weight gains are fairly precise. However, women delivering preterm infants may have been weighed 2 weeks or more before delivery, leading to an underestimation of weight gain and a potential overestimation of the risks of lower weight gains. Underreporting of body mass index among heavier women would lead to an overestimation of weight gain and an overestimation of the benefits of higher weight gains. Fourth, the exclusion of 37% of women owing to missing data resulted in a slightly lower-risk sample of women than the general Johns Hopkins Hospital obstetric population. Fifth, the analyses were based on the weight gains of women delivering before publication of the Institute of Medicine recommendations, but this would affect the results only if weight gain advice influenced the relation between weight gain and pregnancy outcome. These limitations are not unique to our data but reflect the reality of analyzing routinely collected obstetric data, as well as the circumstances under which medical decisions are made regarding the appropriateness of weight gain and the risk of adverse outcomes.

The results indicate that as weight gains increase, the risk of small-for-gestational-age births declines and the risk of large-for-gestational-age births increases, each in an incremental fashion. Thus, recommended weight gains do not confer minimal risks for such births but, rather, represent some acceptably low level of risk for these outcomes. Furthermore, because these risks change over the same range of weight gain (Figure 1), there are direct trade-offs between small- and large-for-gestational-age births to consider when setting weight gain recommendations.

The health implications of small- and large-for-gestational-age births are not equivalent, but both represent suboptimal pregnancy outcomes. Both small- and large-for-gestational-age infants are at increased risk for perinatal mortality.[13] Furthermore, they are at increased risk for fetal distress during delivery and for respiratory distress, hypoglycemia, and hyperbilirubinemia postnatally.[14,15] Large-for-gestational-age infants are more likely to suffer traumas during delivery that may be handicapping or lethal.[16] The birth process and the extrauterine environment are more life threatening for small- and large-for-gestational-age infants, and effective obstetric and perinatal management is crucial to their health and survival. Small- and large-for-gestational-age infants are more frequently delivered by cesarean, and we previously demonstrated a U-shaped relation between size at birth and cesarean delivery at Johns Hopkins Hospital.[17] Therefore, trying to minimize the risk of both small- and large-for-gestational-age births may reduce surgical risks for women. Although it is debatable whether cesarean delivery represents a suboptimal pregnancy outcome, some obstetricians are concerned that higher weight gains will increase cesarean delivery rates unnecessarily.[3,18]

With these considerations in mind, the 3% to 5% likelihood of both small- and large-for-gestational-age births associated with recommended weight gains for women with low



Note. Probabilities were predicted from parameters of the logistic regression models described in the text and presented (as odds ratios) in Table 2. The probabilities are graphed across the observed 10th–90th percentiles of weight gain within each BMI stratum. All covariates were set to their average values or frequencies within each BMI stratum (races pooled), except for maternal race, which was set to 0 or 1 in order to predict race-specific probabilities. See Table 1 for approximate values; exact values are available from the authors. The vertical lines represent the Institute of Medicine recommended weight gain ranges.[1]

FIGURE 1—Probabilities of small-for-gestational-age (SGA) and large-for-gestational-age (LGA) births associated with expected total weight gain at term for Black and White women of varying prepregnancy body mass index (BMI) (kg/m^2).

body mass indexes seems reasonable. To further reduce the risk of small-for-gestational-age births, higher weight gains could be recommended, because the risk of delivering a large-for-gestational-age infant would not increase very much. Among those with average and high body mass indexes, the risks of both types of birth for Black women also seem reasonable and are in line with the risks for women with low indexes. For White women, however, the risks of large-for-gestational-age births are substantially higher, and risks of small-for-gestational-age births are quite low. Thus, weight gain recommendations for White women could perhaps be lowered to diminish the risk of large-for-gestational-age births without greatly increasing the risk of small-for-gestational-age births.

The Institute of Medicine suggested that Black women gain at the upper end of the recommended ranges. We can see why that might be; in this study, Black women were at higher risk of small-for-gestational-age births and lower risk of large-for-gestational-age births than White women. Differences by race were body mass index dependent, because, after adjustment, we found racial differences in risk only among women with average and high body mass indexes. Furthermore, the racial differences were more pronounced for large- than for small-for-gestational-age births. Thus, racial differences in fetal growth relate more to average and overweight Black women being less likely than White women to deliver a large-for-gestational-age baby than to average and thin Black women being more likely than White women to deliver a small-for-gestational-age baby.

Racial differences in the incidence of small- and large-for-gestational-age births among women with average and high body mass indexes were not explained by weight gain or any other factor we examined during analyses. We found no significant interactions between maternal race and weight gain, indicating that the influences of weight gain on the risk of such births were similar for Black and White women. Cogswell et al.[5] also found no interaction between race and weight gain in their analyses of the pregnancy outcomes of more than 53 000 average-weight and overweight women. Our findings do indicate that the effects of body mass index on pregnancy outcomes vary by race. Interestingly, data from Johns Hopkins Hospital reported more than 20 years ago support the findings that (1) the effect of weight gain on pregnancy outcome is similar for Black and White women and (2) the magnitude of the effect of body mass index on pregnancy outcome is smaller for Black women than for White women.[19]

Because race did not influence the risk of small- or large-for-gestational-age births among women with body mass indexes of less than 19.8 kg/m^2, there is little reason to make separate weight gain recommendations by race for these women. Higher weight gains for Black women with average or high body mass indexes could help prevent

218

TABLE 3—Expected Change in Incidence of Small- and Large-for-Gestational-Age Births in Black and White Women Attributable to Changes in Selected Modifiable Risk Factors

	Small for Gestational Age		Large for Gestational Age	
	Black	White	Black	White
Sample incidence, %	7.35	3.97	4.85	14.80
Risk factor change[a]				
No undergain	−1.17 (−16)	−0.44 (−11)	+1.28 (+26)	+2.58 (+17)
No overgain	+0.97 (+13)	+0.60 (+15)	−0.77 (−16)	−2.87 (−19)
No BMI < 19.8 kg/m^2	−0.15 (−2)	−0.64 (−16)	+0.82 (+17)	+3.70 (+25)
No BMI > 26.0 kg/m^2	+0.15 (+2)	+0.32 (+8)	−0.73 (−15)	−1.48 (−10)
No smoking	−2.77 (−38)	−1.10 (−28)	+0.75 (+16)	+1.62 (+11)

Note. Values are based on calculations of attributable risk[12] using exposure data reported in Table 1 and odds ratios reported in Table 2 or available from the authors. BMI = body mass index. The sign of the change indicates the proportion of cases that would be averted (−) or would no longer be averted (+) if there were changes in each modifiable risk factor for Black women and White women, respectively. Undergain and overgain refer to weight gains less than or more than the Institute of Medicine recommended ranges for gestational weight gain.[1]

[a]Expected absolute change (as % of baseline) in incidence.

adverse outcomes, not because the relation between weight gain and pregnancy outcome is different for Black women but because weight gain can be manipulated to equalize the risk of small-for-gestational-age births for Black and White women at acceptably low levels. However, based on our results, Black women with average body mass indexes would have to gain nearly 25 kg to lower their risk of small-for-gestational-age births to that (approximately 3%) of White women gaining 11.5 kg (the lower limit of the recommendation). Similarly, Black women with high body mass indexes would have to gain nearly 18 kg to lower their risk of small-for-gestational-age births to about 5%, the risk for White women gaining only 7.0 kg.

Should weight gain recommendations be raised for Black women? Our results provide little support for advocating that Black women gain at the upper end of the recommendations. First, we observed no difference in risk of adverse outcomes by race among thin women. Second, having average-weight or overweight Black women gain at the upper end of the recommendations is not likely to translate into tangible benefits. For example, the predicted incidence of small-for-gestational-age births for Black women with average body mass indexes gaining at the lower limit of the recommendations (11.5 kg) is 5.7%, whereas the predicted incidence if they gain at the upper end of that range (16 kg, or a 4.5-kg additional gain) is 4.5%. Weight gains for Black women that equalize the risk of small-for-gestational-age births for Black and White women would be high. Although the concomitant increase in the risk of large-for-gestational-age births may be tolerable, such high gains are probably not achievable and may heighten concern about the surgical risks associated with increased maternal weight, as well as postpartum weight retention, obesity, and related health risks.[17,20–22]

Should weight gain recommendations be lowered for White women? This could be justified for women with average or high body mass indexes, whose likelihood of delivering a large-for-gestational-age infant is high and whose likelihood of delivering a small-for-gestational-age infant is low. We hesitate to draw this conclusion from our data alone; rather, we suggest that our approach is a reasonable one to begin addressing this issue. It would be important to conduct similar analyses using larger samples representative of pregnant women in the United States and to separate overweight and very overweight women. The distributions of women by body mass indexes and race in our sample and the representative sample of women participating in the 1988 National Maternal and Infant Health Survey are quite similar[23,24]; however, racial differences in weight gain were somewhat greater in the latter, and the general socioeconomic profile of the Johns Hopkins Hospital sample (regardless of race) implies higher risks for adverse pregnancy outcomes than among US women in general.[7,24]

Given that the relation between weight gain and fetal growth does not seem to vary by race, should we be setting race-specific recommendations for weight gain? Instead of urging Black women to gain different amounts than White women, perhaps we should intensify efforts to illuminate the basis for differential risk of small- and large-for-gestational-age births, which is unexplained by currently measured variables. Furthermore, additional research is needed to understand the relation between body mass index and fetal growth, which does appear to be race specific. □

References

1. Institute of Medicine, Subcommittee on Nutritional Status and Weight Gain during Pregnancy. *Nutrition During Pregnancy*. Washington, DC: National Academy of Sciences; 1990.
2. Parker JD, Abrams B. Prenatal weight gain advice: an examination of the recent prenatal weight gain recommendations of the Institute of Medicine. *Obstet Gynecol*. 1992;79:664–669.
3. Johnson JWC, Longmate JA, Frentzen B. Excessive maternal weight and pregnancy outcome. *Am J Obstet Gynecol*. 1992;167:353–372.
4. Hickey CA, Cliver SP, Goldenberg RL, Kohatsu J, Hoffman HJ. Prenatal weight gain, term birth weight, and fetal growth retardation among high-risk multiparous black and white women. *Obstet Gynecol*. 1993;81:529–535.
5. Cogswell ME, Serdula MK, Hungerford DW, Yip R. Gestational weight gain among average-weight and overweight women—what is excessive? *Am J Obstet Gynecol*. 1995;172:705–712.
6. Edwards LE, Hellerstedt WL, Alton IR, Story M, Himes JH. Pregnancy complications and birth outcomes in obese and normal-weight women: effects of gestational weight change. *Obstet Gynecol*. 1996;87:389–394.
7. Caulfield LE, Witter FR, Stoltzfus RJ. Determinants of gestational weight gain outside the recommended ranges among black and white women. *Obstet Gynecol*. 1996;87:760–766.
8. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. *Obstet Gynecol*. 1996;87:163–168.
9. Kahn HA. Attributable risk. In: *An Introduction to Epidemiologic Methods*. New York, NY: Oxford University Press Inc; 1983.
10. Stewart AL. The reliability and validity of self-reported weight and height. *J Chronic Dis*. 1982;35:295–309.
11. Stevens-Simon C, McAnarney ER, Coulter MP. How accurately do pregnant adolescents estimate their weight prior to pregnancy? *J Adolesc Health Care*. 1986;7:250–254.
12. Rowland ML. Self-reported weight and height. *Am J Clin Nutr*. 1990;52:1125–1133.
13. Williams RL, Creasy RK, Cunningham GC, Hawes WE, Norris FD, Tashiro M. Fetal growth and perinatal viability in California. *Obstet Gynecol*. 1982;59:624–632.
14. Caulfield LE, Haas JD, Belizán JM, Rasmussen KM, Edmonston B. Differences in early postnatal morbidity risk by pattern of fetal growth in Argentina. *Paediatr Perinat Epidemiol*. 1991;5:263–275.
15. Stevenson DK, Hopper AO, Cohen RS, Bucalo LR, Kerner JA, Sunshine P. Macrosomia: causes and consequences. *J Pediatr*. 1982;100:515–520.
16. Boyd ME, Usher RH, McLean FH. Fetal macrosomia: prediction, risks, proposed management. *Obstet Gynecol*. 1983;61:715–722.
17. Witter FR, Caulfield LE, Stoltzfus RJ. Influence of maternal anthropometric status and

birth weight on the risk of cesarean delivery. *Obstet Gynecol.* 1995;85:947–951.
18. Brost BC, Goldenberg RL, Mercer BM, et al. The Preterm Prediction Study: association of cesarean delivery with increases in maternal weight and body mass index. *Am J Obstet Gynecol.* 1997;177:333–341.
19. Eastman NJ, Jackson E. Weight relationships in pregnancy. *Obstet Gynecol Surv.* 1968;23: 1003–1025.
20. Abrams B, Parker J. Overweight and pregnancy complications. *Int J Obes.* 1988;12:293–303.
21. Parker JD, Abrams BA. Differences in postpartum weight retention between black and white mothers. *Obstet Gynecol.* 1993;81:768–774.
22. Taffel SM, Keppel KG, Jones GK. Medical advice on maternal weight gain and actual weight gain: results from the 1988 National Maternal and Infant Health Survey. *Ann N Y Acad Sci.* 1993;678:293–305.
23. Frisbie WP, Biegler M, de Turk P, Forbes D, Pullum SG. Racial and ethnic differences in determinants of intrauterine growth retardation and other compromised birth outcomes. *Am J Public Health.* 1997;87:1977–1983.
24. Keppel KG, Taffel SM. Pregnancy-related weight gain and retention: implications of the 1990 Institute of Medicine guidelines. *Am J Public Health.* 1993;83:1100–1103.

STATISTICS IN MEDICINE, VOL. 17, 341–355 (1998)
Statist. Med., 17, 341–355 (1998)

ESTIMATING THE CAUSAL EFFECT OF COMPLIANCE ON BINARY OUTCOME IN RANDOMIZED CONTROLLED TRIALS

ELS GOETGHEBEUR[1,2]*, GEERT MOLENBERGHS[2] AND JOANNE KATZ[3]

[1] *Applied Mathematics and Informatics, University of Ghent, Krijgslaan 281 -S9, B-9000 Ghent, Belgium*
[2] *Biostatistics, Limburgs Universitair Centrum, B-3590 Diepenbeek, Belgium*
[3] *School of Hygiene and Health, Johns Hopkins University, 615 N Wolfe Street, Rm 5515, Baltimore, MD, 21205, U.S.A.*

SUMMARY

We examine likelihood based methods aimed at analysing the causal effect of actual exposure to drug treatment on a (repeated) binary outcome in two randomized trials with partial compliance. Starting with the univariate compliance summary 'total treatment dose history', we apply a method for ordinal compliance and monotone dose response, proposed by Goetghebeur and Molenberghs. In a short duration trial of blood pressure reduction, this summary leads to meaningful effect estimators. However, in the analysis of a vitamin A trial, this method reaches a boundary solution; the estimated possible benefit from vitamin A for children who did not receive any pills on the treatment arm is zero. In our formulation the number of pills that were taken captures part of the outcome, and the corresponding effect parameters suffer from this confounding. To gain additional insight, we account explicitly for the temporal structure of compliance. We extend the likelihood based methodology for univariate ordered compliance to more dimensional compliance with only a partial order structure on exposure. The randomization assumptions in the causal formulation of Rubin are translated to this setting. We motivate a set of parametric assumptions on the joint distribution of potential outcomes and observed compliance levels and reanalyse the vitamin A trial. Our findings suggest that one capsule of vitamin A had a large impact on mortality during the first 4 months. The greatest reduction in risk was estimated amongst children who received two doses. This supports findings from a vitamin A trial in Ghana and in Nepal. Finally, we discuss extensions of this method, covering uncensored and censored grouped survival data. © 1998 John Wiley & Sons, Ltd.

1. INTRODUCTION

In a typical clinical trial, patients are randomized to one specified dosing strategy of treatment A or treatment B over a period of time. The standard intention-to-treat analysis compares the effect of both treatments at an average in trial exposure level. The limitations of an analysis which ignores compliance levels have received growing concern in the medical and statistical literature; see for instance references 1–7. One has generally recognized the need for complementary analyses which are aimed at estimating the causal effect of exposure at the levels that were

* Correspondence to: Els Goetghebeur, Applied Mathematics and Informatics, University of Ghent, Krijgslaan 281-S9, B-9000 Ghent, Belgium. e-mail: els.goetghebeur@rug.ac.be

Contract grant sponsor: United States Agency for International Development
Contract grant number: DAN-0045-A-5094
Contract grant sponsor: Task Force Sight and Life of Roche, Basel

CCC 0277–6715/98/030341–15$17.50
© 1998 John Wiley & Sons, Ltd.

actually achieved. To this end, experimental and observational data must be combined in one analysis.

Motivated by two randomized controlled trials, this paper develops a missing data approach to study the effect of simple partial compliance patterns which nevertheless contain some key ingredients of more general patterns. Specifically, we analyse a trial on blood pressure reduction and a trial studying the effect of vitamin A on infant mortality.[8] First a univariate, discrete, ordered summary of compliance like grouped 'percentage of dose taken' is considered, and the approach of reference 9 applied to this measure. The second analysis faces the fact that partial compliance on the univariate ordered scale represents in part the survival outcome; children who die during the study do not receive the pills scheduled from then onwards. To understand the possible effects of partial compliance better, we need to distinguish between vitamin intake over the different scheduled times.

The examples are introduced in Section 2. Section 3 introduces the general problem and a set of causal questions. In Section 4 a totally ordered compliance set is introduced and a monotone exposure-response model is applied to the two examples. In Section 5 we extend the method to exposures at different points in time and reanalyse the vitamin A trial. Section 6 discusses extensions of the proposed approach.

2. TWO EXAMPLES

2.1. A trial on blood pressure reduction

The first example is drawn from a relatively small trial conducted by the Janssen Research Foundation, with very detailed measures of compliance. After an initial run-in period, patients are randomized over one of two treatments or placebo, each prescribed at one tablet to be taken daily. Blood pressure measures are taken at regular intervals, including at the beginning and at the end of the active treatment period. Compliance measures were based on a medication event monitoring system (MEMS; Apex Corporation.)* We consider the percentage of prescribed dose which was taken over the active treatment period as the compliance dimension of interest and record whether 'treatment' has failed to control blood pressure $Y_i = 1$ or not, $Y_i = 0$. The treatment is called successful if either the patient has gone down from a baseline diastolic blood pressure of $\geqslant 95$ mmHg to $\leqslant 90$ mmHg or has achieved a 10 per cent reduction in diastolic blood pressure from baseline. The compliance distribution is categorized into three levels: less than 40 per cent of prescribed dose taken (level 0); between 40 per cent and 95 per cent (level 1), and more than 95 per cent (level 2). We focus on one treatment arm and the placebo arm; the data are summarized in Table I.

2.2. A vitamin A trial

Sommer and Zeger[8] analyse a vitamin A trial conducted in rural Indonesia. The aim of the trial is to investigate whether a supplement of high dose vitamin A scheduled on two occasions, 6 months apart, can reduce childhood mortality. Villages were randomized over treatment or control, where for ethical reasons the control arm did not receive any treatment. Eligible children

* MEMS, a relatively novel technique for compliance measuring which uses an electronic pillbox; within the child-proof closure of the MEMS bottles, a microprocessor records the dates and times of each opening

 © 1998 John Wiley & Sons, Ltd.

222

Table I. Categorized blood pressure data

	Treatment group	
	$Y_i^T = 0$	$Y_i^T = 1$
$C_i^T = 0$	4	4
$C_i^T = 1$	8	6
$C_i^T = 2$	21	12
	Placebo group	
	$Y_i^P = 0$	$Y_i^P = 1$
$C_i^P = 0$	6	2
$C_i^P = 1$	2	10
$C_i^P = 2$	10	23

were enumerated first (at day 0 say) and in the treatment group they turned out to get the first pill by month 5 and the second pill between months 6 and 8. Follow-up ends at month 12 (after enumeration) in both groups. The outcome of interest is whether children survive or not.

The trial was designed to evaluate the Indonesian government programme and it was deemed useful to know whether just one capsule causes an effect, whether such effect is delayed and whether additional benefit is obtained from a cumulative dose. In this respect, an intention-to-treat analysis can provide an asymptotically unbiased test of the null hypothesis of no effect of the vitamin A programme as is, but it cannot produce an estimator of a parameter of interest to the secondary questions.

We ignore any geographical, familial clustering as in reference 8 but refine the binary compliance summary, retaining the number of high dose vitamin A pills children received: 0, 1, or 2. Note that no placebo was given and less than half a per cent of the control children said they had received vitamin A outside the programme; their dose will be ignored in our analysis. The analyses we will discuss rely on outcomes averaged over observed compliance levels in the control group. We analyse an updated version of the database, previously analysed in reference 8; whereas reference 8 considered only those children who survived at least four months after enumeration, we will include all enumerated children and classify them as 'non-compliant' when they had no positive record of capsule receipt. The data are shown in Table V.

3. FORMALIZING THE PROBLEM

Consider subjects i ($i = 1, \ldots, N$) who are randomized over a fixed dose of treatment ($R_i = \mathrm{T}$) or placebo/control ($R_i = \mathrm{P}$). For subject i on treatment, a measure of treatment-compliance level $\mathbf{C}_i^T$ is recorded along with response $\mathbf{Y}_i^T$. Likewise, in the placebo arm, placebo-response $\mathbf{Y}_i^P$ is observed, and if a placebo is given, also placebo-compliance $\mathbf{C}_i^P$. Different treatment-compliance levels imply different levels of exposure to the experimental therapy, and we are interested in the 'causal' effect of this post-randomization variable.

In the vitamin A trial, no placebo was given, but also in the blood pressure example, no interesting questions pertain to the amount of placebo taken. In addition, in both trials one can

© 1998 John Wiley & Sons, Ltd.

223

safely assume that subjects on the placebo arm have no access to the experimental therapy. Therefore, in our further development we will ignore $\mathbf{C}_i^{\mathrm{P}}$ and from now on, write $\mathbf{C}_i$ for $\mathbf{C}_i^{\mathrm{T}}$.

Hence, for any subject i, either $\mathbf{Y}_i^{\mathrm{P}}$ or $(\mathbf{C}, \mathbf{Y}_i^{\mathrm{T}})$ is observed, and we can consider the unobserved vector as a latent variable representing what would have been observed had the patient been randomized to the other arm. The causal effect of *treatment assigned* can then be defined per subgroup of patients who achieved a given level of exposure as:

$$E(\mathbf{Y}_i^{\mathrm{T}} - \mathbf{Y}_i^{\mathrm{P}} \mid \mathbf{C}_i = k). \tag{1}$$

When $\mathbf{Y}^{\mathrm{T}} - \mathbf{Y}^{\mathrm{P}}$ varies over different compliance levels g, this is consistent with the assumption that it is the actual level g that causes the shift in response. However, no proof of such causality follows from the analysis.

The key estimation problem for (1) stems from the lack of joint observations on $(\mathbf{Y}_i^{\mathrm{P}}, \mathbf{C}_i)$, but one can rely on *Assumption I: the Randomization Assumption*:

$$(\mathbf{Y}_i^{\mathrm{P}}, \mathbf{C}_i, \mathbf{Y}_i^{\mathrm{T}}) \perp R_i. \tag{2}$$

Even though the tri-variate vector is never jointly observed, (2) must hold when treatment is properly randomized. As a result, the observed $(\mathbf{Y}_i^{\mathrm{P}} \mid R_i = \mathrm{P})$ in the placebo group has the same distribution as the unobserved $(\mathbf{Y}_i^{\mathrm{P}} \mid R_i = \mathrm{T})$ in the treatment group. Similarly for the observed and unobserved pairs $(C_i, \mathbf{Y}_i^{\mathrm{T}} \mid R_i)$.

To allow for a further causal formulation along the lines of references 10 and 11, we need extra notation. Let the observed compliance variable $\mathbf{C}_i$ take values in the set $\mathscr{G}$. Then, any $\mathbf{g} \in \mathscr{G}$ indicates a possible (but for an individual not necessarily achieved) active treatment scenario. With $\mathbf{g} = 0$ we indicate the absence of active treatment, but otherwise the general trial conditions remain. For subject i, the response to each possible level g is denoted $Y_i^{\mathbf{g}}$, a potential outcome which is only observed for $\mathbf{C}_i = \mathbf{g}$, when $R_i = \mathrm{T}$.

Causal questions of interest include:

(i) $E[\mathbf{Y}_i^{\mathbf{g}} - \mathbf{Y}_i^{\mathrm{P}} \mid \mathbf{C}_i = \mathbf{g}]$, the expected benefit from treatment level g in the subgroup who actually receives level g under the trial conditions. This is the same parameter as defined in (1).

(ii) $E[\mathbf{Y}_i^{100\%} - \mathbf{Y}_i^{\mathrm{P}} \mid \mathbf{C}_i = \mathbf{g}]$, the expected benefit from 'punctual dosing' (100 per cent exposure) among patients who took active dose g on the treatment arm.

(iii) $E[\mathbf{Y}_i^{100\%} - \mathbf{Y}_i^{\mathrm{P}}]$, the efficacy parameter, expected benefit from 'punctual' dosing over the entire population.

Complete observations on $(\mathbf{Y}_i^{\mathbf{g}}, \mathbf{C}_i; \mathbf{g} \in \mathscr{G})$, would provide enough information to answer the questions above. Given the incomplete data formulation, extra assumptions are required. Assumptions which reflect the design of a class of trials represented by our two examples are developed in Sections 3 and 5.

4. MONOTONE DOSE RESPONSE

Some progress can be made when considering an ordinal treatment-compliance history, C, and binary outcomes, Y, failure (1) or success (0). The set of possible compliance levels is then denoted $\mathscr{G} = \{0, 1, \ldots, G\}$. Having an ordered compliance scale is at first sight quite restrictive, but most univariate compliance measures of interest, for example, percentage of dose received, number of drug holidays, total time without therapeutic coverage, total time in rebound phase, can easily be categorized in this way.

 © 1998 John Wiley & Sons, Ltd.

224

We consider next two additional assumptions which increase the number of estimable parameters. *Assumption II*: *monotone treatment-dose–response relationship* holds if any failure that happened at exposure level g, would also have happened at lower exposure level g'. Formally:

$$Y_i^g = 1 \Rightarrow Y_i^{g'} = 1 \quad \text{if} \quad g > g'. \tag{3}$$

In the blood pressure trial this implies that potentially increasing the actual drug level intake would not increase the blood pressure; in the vitamin A trial possibly taking more vitamin A would not cause death. As a result, the distribution of (C_i, Y_i^g) is fully characterized by $(G+1) \times (G+2) - 1$ parameters. To see this clearly, consider the one-to-one relationship between (C_i, Y_i^g) and the pair (C_i, T_i), where T_i indicates the lowest exposure level (threshold) at which success is potentially reached, and $T_i = G + 1$ when failure occurs even at the highest exposure level G.

Further, we introduce *Assumption III*: *a placebo link assumption* $Y_i^0 = 0 \Leftrightarrow Y_i^P = 0$. That is, potential response to zero dose of experimental treatment equals the response under control conditions. This assumption will allow our estimation procedure to exploit the randomization assumption as it implies

$$\Pr(Y_i^P = 0 \mid R_i = \mathrm{P}) = \sum_g \Pr(Y_i^0 = 0 \mid R_i = \mathrm{T}, C_i = g) \Pr(C_i = g \mid R_i = \mathrm{T}).$$

Assumptions I–III yield $(G+1) \times 2$ estimable treatment parameters from the $(G+1) \times (G+2) - 1$ characterizing the joint distribution of (C_i, T_i) of which $(G+1) \times 2 - 1$ follow directly from the observed treatment table and an extra parameter is obtained through Assumption III and the placebo data. This implies that extra assumptions are needed to estimate the full joint distribution of C_i and Y_i^g for all $g \in \mathcal{G}$. However, it can be shown,[8] that if only two compliance levels are considered, that is, $G = 1$, all probabilities $E(Y_i^P \mid C_i = g)$ are identifiable. For $G > 1$ unidentifiability remains and extra assumptions have to be made, if one wants to estimate (1).

The observations $(C_i = g, Y_i^T = 0)$ translate into interval censored values of T_i: for individuals with observed compliance g we know either $T_i \leqslant g$ or $T_i > g$. This censoring is ignorable in the sense of Heitjan and Rubin: [12] all patients with $C_i = g$ have probability 1 of being right (or left) censored at g, no matter how much bigger (smaller) than g, T_i truly is reference 12. Hence, to estimate the cell probabilities $\mu_{gl} = \Pr(C_i = g, T_i = l)$ we fit a parametric model to the data which involves at most $(G+1) \times 2$ parameters. For instance, when the choice is made to keep the $G + (G+1)$ marginal probabilities $\sum_g \mu_{gl}, \sum_l \mu_{gl}$, unrestricted, one can further estimate a one-parameter association measure.

For our examples, we fitted a Dale model[13] with $G + G + 1$ parameters describing the unrestricted marginal probabilities $\sum_g \mu_{gl}, \sum_l \mu_{gl}$ and one extra parameter expressing a constant global odds ratio as a single association parameter. Specifically, the marginal probabilities are modelled using cumulative logits:

$$\eta_{1g} = \ln\left(\frac{\Pr(C_i > g)}{\Pr(C_i \leqslant g)}\right), \quad 0 \leqslant g \leqslant G - 1 \tag{4}$$

$$\eta_{2l} = \ln\left(\frac{\Pr(T_i > l)}{\Pr(T_i \leqslant l)}\right), \quad 0 \leqslant l \leqslant G \tag{5}$$

© 1998 John Wiley & Sons, Ltd.

225

Table II. Compliance and threshold levels in the treatment arm of the blood pressure trial

	Estimated numbers					Estimated dose response		
	$T_i=0$	$T_i=1$	$T_i=2$	$T_i>2$		$\Pr(T_i=0)$	$\Pr(T_i\leqslant 1)$	$\Pr(T_i\leqslant 2)$
$C_i=0$	4·00	1·17	1·41	1·42	$C_i=0$	0·50	0·65	0·82
$C_i=1$	5·83	2·17	2·88	3·12	$C_i=1$	0·42	0·57	0·78
$C_i=2$	8·85	4·48	7·67	12·00	$C_i=2$	0·27	0·40	0·64

whilst the global odds ratios ψ_{gl}:

$$\eta_{3gl}=\ln\psi_{gl}=\ln\left(\frac{\Pr(C_i>g,T_i>l)\Pr(C_i\leqslant g,T_i\leqslant l)}{\Pr(C_i>g,T_i\leqslant l)\Pr(C_i\leqslant g,T_i>l)}\right),\quad 0\leqslant g\leqslant G-1, 0\leqslant l\leqslant G \tag{6}$$

are assumed constant over g and l: $\psi=\psi_{gl}$. The implication of a constant global odds ratio is that the dose–response curves, involving conditional probabilities $d_{gl}=\sum_{s=0}^{l}\mu_{gs}/\mu_{g+}$, are ordered: for $g\leqslant g'$, $d_{gl}\leqslant d_{g'l}\Leftrightarrow\psi\geqslant 1$. A proof of this is given in the Appendix.

Alternatively, one could spend fewer degrees of freedom on the marginal probabilities, and more on the association. This might allow for crossing dose–response curves. The option for our parameterization is motivated by its relative simplicity and the possibility to establish a link with an underlying latent Plackett variable.[14]

Details on model fitting with incomplete data can be found in reference 15.

4.1. Results for the blood pressure trial

Table II shows estimated numbers in the completed treatment table for the blood pressure example based on a Dale model with constant global odds ratio. For instance, of the 8 subjects with compliance level 0, 1·17 are estimated to have a successful response under the potential exposure level 1, but not at exposure level 0.

A graphical representation of the estimated dose–response curves, that is, the cumulative distribution function of the threshold variable, is given in Figure 1 per compliance subgroup. This picture also shows average placebo response and asymptotic 95 per cent confidence intervals.

We have discussed that the imposed model accounts for non-crossing curves. The fit suggests that patients who are least compliant have a better zero dose prognosis than those who are taking most drugs, thus suggesting that a naive C_i/Y_i^{P} association analysis would underestimate the effect of dose.

Regarding causal question (ii) of Section 3, the estimated percentage of patients in each group that would go from unsuccessful outcome to success when taking the full dose instead of none is estimated to be comparable over the different compliance levels: 32 per cent, 36 per cent and 37 per cent in compliance group 0, 1 and 2, respectively. In response to question (i) of Section 3, the estimated proportion of patients that have achieved blood pressure control at the observed compliance level, but would not have achieved it at the zero level, are 16 per cent of the level 1 compliers and 37 per cent of the level 2 compliers. In summary, the point estimates suggest this treatment is quite effective and more so at the higher dose level. However, numbers in the table are quite small, particularly when we consider compliance subgroups and realize that the dose–response curves for them are based on very coarse data. It need therefore be no surprise that for the estimates of the gain from treatment achieved by level 1 compliers and level 2 compliers,

 © 1998 John Wiley & Sons, Ltd.

226

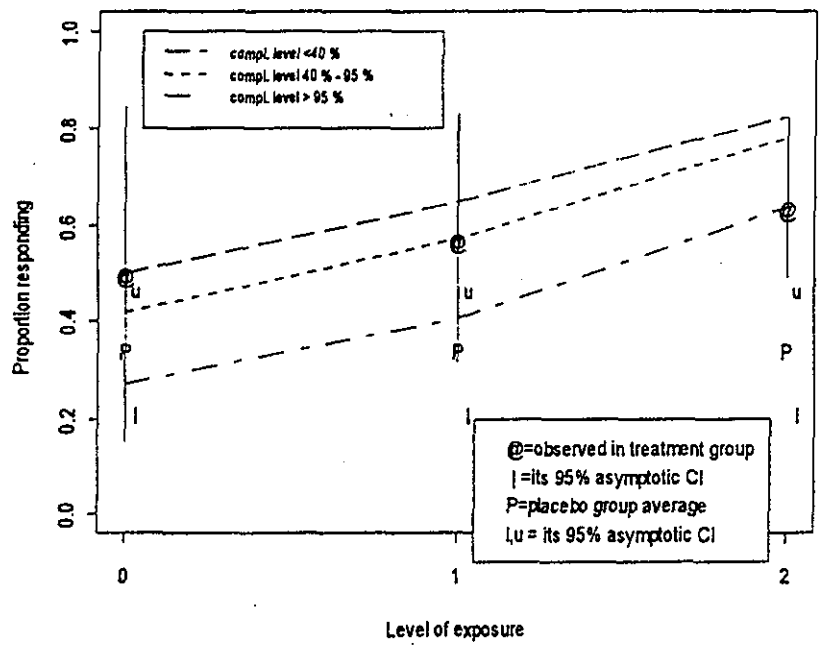


Figure 1. Estimated dose–response curves for the blood pressure trial

the delta method derives from the likelihood based covariance matrix standard errors as large as 0·57 and 0·52, respectively, indicating that the 95 per cent confidence interval covers the whole range of possible outcomes. Clearly, the estimates discussed here are only of a descriptive quality; no 'significant' effects are found under the model from this data set.

The estimates above follow from the data, assuming a class of Dale models with *constant* global odds ratio holds. To explore the extent to which these estimates rely on that specific association constraint, we have also fitted some other models for the associations in the tables. The number of odds ratio functions that determine a valid probability distribution function and yield the same data log-likelihood value is infinite. We have scanned a grid of such functions and present here two rather extreme examples representing a considerable gradient in increasing and decreasing odds, respectively. Note that a whole set of still more extreme choices does no longer lead to genuine probability mass functions. Arguably, a formal sensitivity approach would have to be developed in order to fully assess the impact of changing the association model on the estimated treatment effects. This is the topic of ongoing research.

Instead of imposing $\psi_{gl} = \psi$ $(g = 0, 1; l = 0, 1, 2)$ we imposed $\psi_{gl} = c_{gl}\psi (g = 0, 1; l = 0, 1, 2)$ for

$$\mathbf{c}_1 = \begin{pmatrix} 1{\cdot}00 & 1{\cdot}40 & 1{\cdot}80 \\ 1{\cdot}50 & 2{\cdot}10 & 2{\cdot}70 \end{pmatrix} \quad \text{and} \quad \mathbf{c}_2 = \begin{pmatrix} 1{\cdot}00 & 0{\cdot}60 & 0{\cdot}20 \\ 0{\cdot}70 & 0{\cdot}42 & 0{\cdot}14 \end{pmatrix}.$$

For all examined association measures, the 'estimated placebo response' for 0-level compliers was better than for 2-level compliers. The 1-level group had a more variable behaviour ranging from an estimated placebo response slightly better than the 0-level compliers, with full exposure response somewhat worse than that of 0-level compliers, to an estimated placebo response

© 1998 John Wiley & Sons, Ltd.

237

Table III. Compliance and threshold levels in the blood pressure trial: estimated dose–response curves for different association assumptions

	$\mathbf{c}_1 = \begin{pmatrix} 1{\cdot}0 & 1{\cdot}4 & 1{\cdot}8 \\ 1{\cdot}5 & 2{\cdot}1 & 2{\cdot}7 \end{pmatrix}$		
	$\Pr(T_i = 0)$	$\Pr(T_i \leqslant 1)$	$\Pr(T_i \leqslant 2)$
$C_i = 0$	0·50	0·66	0·91
$C_i = 1$	0·55	0·57	0·90
$C_i = 2$	0·21	0·32	0·64

	$\mathbf{c}_2 = \begin{pmatrix} 1{\cdot}00 & 0{\cdot}60 & 0{\cdot}20 \\ 0{\cdot}70 & 0{\cdot}42 & 0{\cdot}14 \end{pmatrix}$		
	$\Pr(T_i = 0)$	$\Pr(T_i \leqslant 1)$	$\Pr(T_i \leqslant 2)$
$C_i = 0$	0·50	0·60	0·69
$C_i = 1$	0·28	0·57	0·72
$C_i = 2$	0·32	0·44	0·64

slightly worse than the 2-level compliers, with full exposure response somewhat better than that of 2-level compliers. The estimated dose–response curves for these two models are given in Table III.

In conclusion, although these models are indistinguishable in terms of model fit, while part of the interpretation tends to change, the global suggestion remains throughout that 0-level compliers have better treatment-free prognosis than 2-level compliers and hence that a simple dose–response association would tend to underestimate the real effect of dose. We point out that the interpretation depends on the placebo link assumption which in this case means that no actual benefit is obtained from a regime with less than 40 per cent compliance. Finally, the constant odds ratio model is the simplest model and therefore our preferred model in this exercise.

4.2. Results for the vitamin A trial

When fitting the constant odds ratio Dale model to the data described in Section 2.2, one finds that a number of estimated probabilities μ_{gl} fall on the boundary of the parameter space. In particular, $\hat{\mu}_{0,1}$ and $\hat{\mu}_{0,2}$ are zero, implying that children who received no pills, would not have benefited from 1 or 2 pills if those had been received at the scheduled time. At first sight, this is a disappointing and unlikely result which might suggest a wrong model. However, children who died in the first 2–3 months after study may not have received any pills and are likely to constitute a substantial portion of the deaths in that compliance category. Certainly, a child's death before the first pill was due would not have been helped by 1 or 2 pills delivered after death. Less dramatically, it may indicate that the very sick children are 'self-selected' into the low compliance category and that they cannot be helped by the mild treatment of a high vitamin A dose. The need for a method which specifically accounts for shrinking populations over time suggests a multi-dimensional compliance measure.

 © 1998 John Wiley & Sons, Ltd.

228

5. BINARY COMPLIANCE OVER TWO DISTINCT POINTS IN TIME

We now explicitly consider repeated 'all or nothing' indicators of treatment intake over time, rather than ordinal summaries. For simplicity, let us focus on just two doses to be taken: an initial one, at t_0 which is the time of study entry and the start of period I. Another dose is due at t_1, which ends period I and indicates the start of period II which finishes at t_2. The components of $\mathbf{C}_i = (C_{1i}, C_{2i})$, now indicate reception of the dose over periods I and II, respectively. Further, the components of $\mathbf{Y}_i = (Y_{1i}, Y_{2i})$ indicate the occurrence of an event (1 if it occurred, 0 otherwise) by the end of periods I and II, respectively. We assume that the final trial result is reached as soon as the even occurs (see a survival outcome). Hence there are only three possible outcomes: $\mathbf{Y}_i \in \{(1, 1), (0, 1), (0, 0)\}$. These will also be denoted observed outcomes 1, 2 and 3, respectively referring to their simple rank order.

The timing of a drug or therapy has the following causal implication: a different (potential) treatment scenario for tomorrow cannot be the cause of a change in today's response. Formally, the *Assumption IV*: *chronology of exposure*, states: at the possible treatment levels (g_1, g_2) and $(g_1, 1 - g_2)$: $Y_{1i}^{(g_1, g_2)} = Y_{1i}^{(g_1, 1-g_2)}$.

Because there is only a partial order on the set of possible exposure levels, the *monotone exposure-response assumption* becomes *Assumption V*:

$$Y_{1i}^{(g_2, m_2)} \leqslant Y_{1i}^{(g_1, m_1)} \quad \text{and} \quad Y_{2i}^{(g_2, l_2)} \leqslant Y_{2i}^{(g_1, l_1)}, \quad g_2 \geqslant g_1;\ m_1;\ m_2;\ l_2 \geqslant l_1.$$

5.1. Joint causal distribution

Following the logic of Section 4, consider jointly for each subject i the compliance level $\mathbf{C}_i$ and the outcomes to the four possible exposure levels: $(Y_i^{00}, Y_i^{01}, Y_i^{10}, Y_i^{11})$. Ignoring the chronology and monotone dose response assumptions, the four-variate variable would take $3^4 = 81$ values, to be combined with four observed compliance levels in a contingency table. The observed data, consisting of only three possible outcomes at each compliance level, determine an extremely coarse partition of the cells. However, the assumptions on chronology of exposure and monotone exposure-response reduce the set of possible joint outcomes in the treatment arm to just 10. The corresponding contingency table is given in Table IV.

The entries 1, 2 and 3 place *observed* outcomes in unions of cells in each row of the table. For instance, a subject with observed treatment compliance (1, 0) and outcome 3 = (0, 0) yields a data point in the union of cells $\bigcup_{r=3, c\in\{1,2,4,7\}}$ where r is the cell's row number and c its column number.

For the 39 degrees of freedom in Table IV there are only 11 observed data degrees of freedom. Further assumptions are needed for estimation, foremost assumptions which reflect the randomized design.

Assumption VI: *repeated placebo link*; as before, we choose to link response in the control group as a whole (combined over observed compliance levels) with response to the zero active treatment dose $g = (0, 0)$ (potentially) in the treatment arm as a whole; we assume that with active dose level zero, patients have no expected change in response from under the control (or placebo) regime. Formally:

$$Y_{1i}^{0g_2} = 0 \Leftrightarrow Y_{1i}^{\mathrm{P}} = 0 \tag{7}$$

and

$$Y_{2i}^{00} = 0 \Leftrightarrow Y_{2i}^{\mathrm{P}} = 0. \tag{8}$$

Assumptions (7) and (8) equate the sum of the probabilities associated with each cell in column 1 of Table IV to the probability of outcome 3 in the placebo group as a whole and equate the sums

© 1998 John Wiley & Sons, Ltd.

229

Table IV. Contingency table for possible responses per compliance level in the treatment arm

Possible exposures	Joint outcomes to the four possible exposure levels: 1	2	3	4	5	6	7	8	9	10
$(g_1, g_2) = (0, 0)$	(0, 0)	(0, 1)	(0, 1)	(0, 1)	(0, 1)	(0, 1)	(1, 1)	(1, 1)	(1, 1)	(1, 1)
$(g_1, g_2) = (0, 1)$	(0, 0)	(0, 0)	(0, 0)	(0, 1)	(0, 1)	(0, 1)	(1, 1)	(1, 1)	(1, 1)	(1, 1)
$(g_1, g_2) = (1, 0)$	(0, 0)	(0, 0)	(0, 1)	(0, 0)	(0, 1)	(0, 1)	(0, 0)	(0, 1)	(0, 1)	(1, 1)
$(g_1, g_2) = (1, 1)$	(0, 0)	(0, 0)	(0, 0)	(0, 0)	(0, 0)	(0, 1)	(0, 0)	(0, 0)	(0, 1)	(1, 1)
Compliance										
$\mathbf{C} = (0, 0)$	3	2	2	2	2	2	1	1	1	1
$\mathbf{C} = (0, 1)$	3	3	3	2	2	2	1	1	1	1
$\mathbf{C} = (1, 0)$	3	3	2	3	2	2	3	2	2	1
$\mathbf{C} = (1, 1)$	3	3	3	3	3	2	3	3	2	1

of cell probabilities in columns $\{2, 3, 4, 5, 6\}$ to the probability of outcome 2 in the placebo group. This adds two extra observed degrees of freedom on parameters in Table IV.

In Section 4, for the univariate compliance measure, a modelling approach for ordinal outcomes was applied directly to the table with possible responses to treatment. Here, the simple ordered structure on the set of 10 possible outcomes is lost and the choice of a reasonable parsimonious model for the table is much harder. Therefore, we forgo the characterization of the *joint* outcome to the four possible treatment regimes and estimate instead only the probability of response to each separate treatment regime jointly with the observed compliance level. For $\mathbf{g} = (0, 0)$, this amounts to estimating the probabilities of outcome 1 and $\{2, 3, 4, 5, 6\}$ per observed compliance level. Different partitions appear for different g-levels. Such 'marginal' characterization is sufficient to answer the causal questions in Section 3.

5.2. Model formulation

Consider the following joint model for response to a possible scenario $\mathbf{g} = (g_1, g_2)$ and observed treatment compliance level $\mathbf{C}_i$:

$$\eta_1[\Pr(Y_{1i}^{\mathbf{g}} = 1 \mid \mathbf{C}_i)] = \alpha_0(\mathbf{C}_i) + \alpha_1(\mathbf{C}_i)\, I_{g_1}(1) \tag{9}$$

$$\eta_2[\Pr(Y_{2i}^{\mathbf{g}} = 1 \mid \mathbf{C}_i, Y_{1i}^{\mathbf{g}} = 0)] = \beta_0(\mathbf{C}_i^{\mathrm{T}}) + \beta_1^{01}(\mathbf{C}_i)\, I_{\mathbf{g}}(01) + \beta_1^{10}(\mathbf{C}_i)\, I_{\mathbf{g}}(10) + \beta_1^{11}(\mathbf{C}_i)\, I_{\mathbf{g}}(11)$$

where $I_{\mathbf{g}}(01)$ and $I_{g_1}(1)$ indicate that $\mathbf{g} = (0, 1)$ and $g_1 = 1$, respectively, η_1 and η_2 are link functions like the logit link, and the right hand side represents an estimable (linear) function. We have incorporated the chronology assumption through the appearance of only g_1 in (9): response at t_1 cannot be altered by a *possible* intake over period II. All parameters on the right hand side are initially allowed to depend on $\mathbf{C}_i$, but must be further restricted to be estimable. We will examine the following choice:

$$\alpha_0(\mathbf{C}_i) = \alpha_0(C_{1i})$$

$$\alpha_1(\mathbf{C}_i) = \alpha_1,$$

$$\beta_0(\mathbf{C}_i) = \theta + \alpha_0(C_{1i}),$$

$$\beta_1^{01}(\mathbf{C}_i) = \beta_1^{01}, \beta_1^{10}(\mathbf{C}_i) = \beta_1^{10}, \beta_1^{11}(\mathbf{C}_i) = \beta_1^{11}.$$

 © 1998 John Wiley & Sons, Ltd.

230

With logit link functions, this is interpreted as follows:

(i) $\alpha_0(\mathbf{C}_i) = \alpha_0(C_{1i})$; we do not distinguish between baseline risks over the first period for people with a different compliance behaviour over the second period. We lack this distinguishing information for a survival outcome, basically because we never see compliance over the second period for those who failed in the first period. This constraint is different from the chronology of exposure assumption, which is concerned with effects added on top of $\alpha_0(\mathbf{C})$. If the outcome vector $\mathbf{Y}$ with binary components could take on all four possible combinations of 0 and 1, we would not be required to make any constraint on α_0, and could still obey Assumption IV.

(ii) A proportional odds model is assumed to link baseline risks (corresponding to no exposure: $\mathbf{g} = (0, 0)$) at different observed compliance levels over the two periods: $\beta_0(\mathbf{C}_i) = \theta + \alpha_0(\mathbf{C}_i)$.

(iii) The causal effect of a possible drug intake at the first time point is assumed equal for (or an average is measured over) each observed compliance category: $\alpha_1(\mathbf{C}_i) = \alpha_1$, no compliance by exposure interaction has been included.

(iv) Likewise at the second time point, the added effect of a possible exposure is not allowed to depend on the compliance subgroup: $\beta_1^{01}(\mathbf{C}_i) = \beta_1^{01}$, $\beta_1^{10}(\mathbf{C}_i) = \beta_1^{10}$ and $\beta_1^{11}(\mathbf{C}_i) = \beta_1^{11}$.

Note, however, that we are allowing for a main effect of the possible exposures over periods I and II, as well as an interaction effect on response over period II.

5.3. Maximum Likelihood Estimation

Let $\pi_{00}^{\mathrm{T}}, \pi_{01}^{\mathrm{T}}, \pi_{10}^{\mathrm{T}}$ indicate the probability of falling into treatment compliance categories $\mathbf{C} = (0, 0), (0, 1)$ and $(1,0)$, respectively. Furthermore, let $E(x) = \exp(x)/1 + \exp(x)$ and N_g^{T} be the number of patients on the treatment arm in compliance category g. Then, with $\pi_{11}^{\mathrm{T}} = 1 - \pi_{00}^{\mathrm{T}} - \pi_{01}^{\mathrm{T}} - \pi_{10}^{\mathrm{T}}$, Assumption IV implies:

$$m_1^{\mathrm{P}} = \Pr(Y_1^{\mathrm{P}} = 1) = \sum_j E[\alpha_0(j)]\pi_j^{\mathrm{T}},$$

$$m_2^{\mathrm{P}} = \Pr(Y_2^{\mathrm{P}} = 1, Y_1^{\mathrm{P}} = 0) = \sum_j E[\theta + \alpha_0(j)]\{1 - E[\alpha_0(j)]\}\pi_j^{\mathrm{T}}$$

where j ranges over all observed compliance levels. This gives the following contribution of the placebo arm to the log-likelihood:

$$\sum_{i:R_i=\mathrm{P}} Y_{1i}^{\mathrm{P}} \log(m_1^{\mathrm{P}}) + (1 - Y_{1i}^{\mathrm{P}})\, Y_{2i}^{\mathrm{P}} \log(m_2^{\mathrm{P}}) + (1 - Y_{1i}^{\mathrm{P}})\,(1 - Y_{2i}^{\mathrm{P}}) \log(1 - m_1^{\mathrm{P}} - m_2^{\mathrm{P}}).$$

With $I_{Ci}(00)$ indicating that $\mathbf{C}_i = (0, 0)$, letting $\mathbf{x}'_{1i} = (I_{Ci}(00), I_{Ci}(01), I_{Ci}(10), I_{Ci}(11))$, $\mathbf{x}'_{2i} = (I_{Ci}(01), I_{Ci}(10), I_{Ci}(11))$, $\boldsymbol{\alpha}'_0 = (\alpha_0(0), \alpha_0(0), \alpha_0(1), \alpha_0(1))$ and $\boldsymbol{\beta}'_1 = (\beta_1^{01}, \beta_1^{10}, \beta_1^{11})$, the treatment data contribute:

$$\begin{aligned}
&\sum_{i:R_i=\mathrm{T}} Y_{1i}^{\mathrm{T}}[\boldsymbol{\alpha}'_0 \mathbf{x}_{1i} + \alpha_1 I_{C_{1i}}(1)] - \log\{1 + \exp[\boldsymbol{\alpha}'_0 \mathbf{x}_{1i} + \alpha_1 I_{C_{1i}}(1)]\} \\
&\quad + (1 - Y_{1i}^{\mathrm{T}})\, Y_{2i}^{\mathrm{T}}(\theta + \boldsymbol{\alpha}'_0 \mathbf{x}_{1i} + \boldsymbol{\beta}'_1 \mathbf{x}_{2i}) - (1 - Y_{1i}^{\mathrm{T}}) \log\{1 + \exp[\theta + \boldsymbol{\alpha}'_0 \mathbf{x}_{1i} + \boldsymbol{\beta}'_1 \mathbf{x}_{2i}]\} \\
&\quad + N_{00}^{\mathrm{T}} \log(\pi_{00}^{\mathrm{T}}) + N_{01}^{\mathrm{T}} \log(\pi_{01}^{\mathrm{T}}) + N_{10}^{\mathrm{T}} \log(\pi_{10}^{\mathrm{T}}) + N_{11}^{\mathrm{T}} \log(1 - \pi_{00}^{\mathrm{T}} - \pi_{01}^{\mathrm{T}} - \pi_{10}^{\mathrm{T}}).
\end{aligned}$$

© 1998 John Wiley & Sons, Ltd.

231

Table V. Data on bivariate reception of the vitamin A and grouped survival

C	Month of death 0–4	5–8	9–12	> 12
		Treatment arm		
00	20	13	7	2546
01		11	5	1495
10	6	2	2	550
11		3	3	7704
		Placebo arm		
	42	33	29	11523

Table VI. Results from the vitamin A data, with bivariate compliance

Parameter	Estimated value	Standard error
$\alpha_0(0)$	−5·25	(0·19)
$\alpha_0(1)$	−5·95	(0·31)
θ	−0·16	(0·22)
α_1	−1·28	(0·52)
β_1^{01}	0·49	(0·37)
β_1^{10}	0·48	(0·80)
β_1^{11}	−1·75	(0·65)

Both contributions are then summed and parameter estimation can proceed by, for instance, Newton–Raphson or Fisher scoring. We used the built-in optimizers of S-plus (nlmin) and GAUSS (optmum, which also produces standard errors).

5.4. The vitamin A trial

The pair of indicators of vitamin A reception on both occasions is the treatment compliance measure of interest. Survival is considered over the initial period up to month 5 (exclusive) and then over the period months 5–8. The data are given in Table V. Two of the children who died in the first period had already received 2 pills. This has no direct effect on the baseline risk estimator over the first period, as α_0 depends on C_{1i} only. Following the model, a possible effect of the second pill has only been accounted for over the second period.

For the model developed in Section 5.2, model parameters (and standard errors) are shown in Table VI. We find a significant positive effect of the first vitamin A pill over the first period, $\hat{\alpha}_1 = -1{\cdot}28$, an estimated 72 per cent reduction in odds of death. Over the second time period no significant effect of a single dose has been found, be it received at $t_0(\beta_1^{10})$ or at $t_1(\beta_1^{01})$. However, a significant reduction in risk over the second time period is estimated for the children who received vitamin A on both occasions: $\beta_1^{11} = -1{\cdot}75$, or an estimated reduction in the odds of mortality over the second period by 83 per cent. We note that the baseline risk of children observed to get the first pill, as expressed via $\alpha_0(1)$, is estimated to be somewhat smaller than for those who do not, see $\alpha_0(0)$. However this difference is not significant.

 © 1998 John Wiley & Sons, Ltd.

232

These findings support conclusions from other vitamin A studies. A trial in Ghana[16] linked the four-monthly administration of vitamin A over a period of 2 years to the hazard of mortality. The mortality rate ratio comparing vitamin A treatment with placebo, was estimated after receiving 1, 2, 3, ..., doses, respectively. No adjustment was made for possibly different compliance selection effects in treatment and placebo, and no overall trend of the rate ratio was found over all possible doses actually received. However, the rate ratio after two doses (0·53; 95 per cent CI: 0·38, 0·72) was lower than after one dose (0·99; 95 per cent CI: 0·78, 1·25) with non-overlapping 95 per cent confidence intervals. A further vitamin A study in Nepal,[17,18] studied four-monthly administration of vitamin A over a total period of 12 months. The published intention-to-treat analysis showed a marked effect of vitamin A, with a (not significantly) lower risk ratio over the second term than over the first term. No analysis per dose received was published there.

Nested submodels can further be explored within our frame, guided by likelihood ratios. According to the latter criterion, the model we considered here is virtually identical to the saturated model with placebo link (which has one additional degree of freedom).

6. DISCUSSION

We have examined likelihood based approaches towards estimation of causal parameters in controlled clinical trials with partial compliance and (repeated) binary outcomes. Once a summary of the compliance history has been chosen, the missing data frame allows causal questions to be well defined and the unobserved portions of the data to be clearly recognized. The stages of simplifying the model in order to allow for more precise and useful estimators need to be discussed for each specific example. One needs to recognize that an estimable model for the observed data can be generated by a range of different models for the potential responses. A choice between those cannot be made on the grounds of the data at hand but needs to rely on subject matter knowledge or other external sources of information. To assess robustness of any conclusions to the modelling assumptions imposed, one can perform a sensitivity analysis, scanning a range of different modelling assumptions. We have made some attempt to explore this for our examples. More work on a structured approach for this is ongoing. In an earlier paper, Goetghebeur and Molenberghs[9] have calculated boundaries on some of the parameters of interest.

When the outcome is a *censored* grouped survival time, the likelihood described in the Appendix can be adapted along the lines of the actuarial method. A subject censored within a given time interval may be assumed to be at risk for about half the length of that interval, thus approximating the censoring time distribution by a uniform distribution over the interval. Formally, this means that the linear combination representing the discrete hazard for the subject over that given period is halved in the log-likelihood expression.

When ordered compliance levels at more than two time points and also baseline covariates are available, the approach naturally extends, but more modelling options are available such as a smooth parametric model for zero-exposure risks over time, of baseline risks over different compliance categories, of causal effects of the possible exposure levels, of an interaction between compliance level and exposure effect or of selection and 'causal' effects varying over the baseline covariates. The model builder must continuously make choices in terms of where the degrees of freedom in the data are spent. Different (semi-) parametric modelling options can be explored as an examination of the sensitivity of the conclusions to one or the other. The multiplicity of options calls for a cautious interpretation of these results as hypothesis generating. Nevertheless,

© 1998 John Wiley & Sons, Ltd.

233

we feel they can form a necessary complement to the intention-to-treat estimator which sometimes estimates a parameter of limited practical interest.

APPENDIX: ORDERED DOSE–RESPONSE CURVES AND THE DALE MODEL

We establish that monotone dose–response curves follow under a constant global odds ratio model when $\psi \geqslant 1$. It is sufficient to establish this relationship for adjacent curves ($g' = g + 1$). For arbitrary fixed l, define:

$$A = \sum_{s=0}^{g-1} \sum_{t=0}^{l} \mu_{st} \qquad B = \sum_{s=0}^{g-1} \sum_{t=l+1}^{G+1} \mu_{st}$$

$$C = \sum_{t=0}^{l} \mu_{gt} \qquad D = \sum_{t=l+1}^{G+1} \mu_{gt}$$

$$E = \sum_{t=0}^{l} \mu_{g't} \qquad F = \sum_{t=l+1}^{G+1} \mu_{g't}$$

$$K = \sum_{s=g'+1}^{G} \sum_{t=0}^{l} \mu_{st} \qquad L = \sum_{s=g'+1}^{G} \sum_{t=l+1}^{G+1} \mu_{st}.$$

Now

$$d_{gl} \leqslant d_{g'l} \Leftrightarrow \frac{E}{E+F} \geqslant \frac{C}{C+D} \Leftrightarrow \frac{ED}{FC} \geqslant 1.$$

Since we assume a global odds ratio model

$$\frac{K(F+D+B)}{L(E+C+A)} = \frac{(K+E)\ (D+B)}{(F+L)\ (C+A)} = \frac{(K+E+C)B}{(L+F+D)A} = \psi.$$

Solving for F, D, B simultaneously yields

$$F = \frac{\psi LE(K+E+C+A)}{K[K+E+\psi(C+A)]}$$

$$D = \frac{\psi LC(K+\psi(E+C+A)](K+E+C+A)}{K(K+E+C+\psi A)\ [K+E+\psi(C+A)]}$$

and hence

$$\frac{ED}{FC} = \frac{K+\psi(E+C+A)}{K+E+C+\psi A} \geqslant 1 \Leftrightarrow \psi \geqslant 1.$$

ACKNOWLEDGEMENTS

The authors thank Keith West, Scott Zeger and statisticians from the Janssen Research Foundation for their help and for making the data available. Support for the vitamin A trial was provided under the Cooperative Agreement DAN-0045-A-5094 from the Office of Nutrition, United States Agency for International Development, and Task Force Sight and Life of Roche, Basel.

 © 1998 John Wiley & Sons, Ltd.

234

REFERENCES

1. Urquhart, J. and Chevalley, C. 'Impact of unrecognized dosing errors on the cost and effectiveness of pharmaceuticals', *Drug Information Journal*, **22**, 363–378 (1988).
2. Cramer, J. A. and Spilker, B. (ed.) *Non-compliance in Clinical Trials*, Raven Press, New York, 1992.
3. Goetghebeur, E. and Pocock, S. 'Statistical issues in allowing for non-compliance and withdrawal', *Drug Information Journal*, **27**, 837–845 (1993).
4. Robins, J. M. and Greenland, S. 'Adjusting for differential rates of pcp prophylaxis in high-versus low-dose azt treatment arms in an aids randomized trial', *Journal of the American Statistical Association*, **89**, 737–749 (1994).
5. Salsburg, D. 'Intent to treat: the reductio ad absurdum that became gospel', *Pharmacoepidemiology and Drug Safety*, **3**, 329–335 (1994).
6. Sheiner, L B. and Rubin, D. B. 'Intention to treat analysis and the goals of clinical trials', *Clinical Pharmacology and Therapeutics*, **57**, 6–15 (1995).
7. Angrist, J. D. Imbens, G. W. and Rubin, D. B. 'Identification of causal effects using instrumental variables', with rejoinder, *Journal of the American Statistical Association*, **91**, 444–471 (1996).
8. Sommer, A. and Zeger, S. L. 'On estimating efficacy from clinical trials', *Statistics in Medicine*, **10**, 45–52 (1991).
9. Goetghebeur, E. and Molenberghs, G. 'Causal inference in a placebo-controlled clinical trial with binary outcome and ordered compliance', *Journal of the American Statistical Association*, **91**, 928–934 (1996).
10. Rubin, D. B. 'Inference and missing data', *Biometrika*, **63**, 581–592 (1976).
11. Holland, P. W. 'Statistics and causal inference', *Journal of the American Statistical Association*, **81**, 945–970 (1986).
12. Heitjan, D. F. and Rubin, D. B. 'Ignorability and coarse data', *Annals of Statistics*, **19**, 2244–2253 (1991).
13. Dale, J. 'Global cross-ratio models for bivariate discrete ordered responses', *Biometrics*, **42**, 909–917 (1986).
14. Plackett, R. L. 'A class of bivariate distributions', *Journal of the American Statistical Association*, **81**, 945–970 (1986).
15. Molenberghs, G. and Goetghebeur, E. 'Simple fitting algorithms for incomplete categorical data', *Journal of the Royal Statistical Society, Series B*, **59**, 401–414 (1997).
16. Ghana Vast Study Team. 'Vitamin A supplementation in northern Ghana: effects on clinic attendances, hospital admissions, and child mortality', *Lancet*, **342**, 7–12 (1993).
17. West, K. P., Pokhrel, R. P., Katz, J., LeClerq, S. C., Khatry, S. K., Shrestha, S. R., Pradhan, E. K., Tielsch, J. M., Pandey, M. R., Sommer, A. 'Efficacy of Vitamin A in reducing preschool child mortality: a randomized double-masked community trial in Nepal', *Lancet*, **338**, 67–71 (1991).
18. Pokhrel, R. P., Khatry, S. K., West, K. P., Shrestha, S. R., Katz, J., Pradhan, E. K., LeClerq, S. C., Sommer, A. 'Sustained reduction in child mortality with Vitamin A in Nepal', *Lancet*, **343**, 1368–1369 (1994).

© 1998 John Wiley & Sons, Ltd.

235

Agreement Between Clinical Examination and Parental Morbidity Histories for Children in Nepal

by Joanne Katz,* ScD, Keith P. West Jr,* DrPH, Steven C. LeClerq.* MPH, M. D. Thapa,** OA, Subarna K. Khatry,** FRCS, Sharda Ram Shresta.** MPH, Elizabeth Kimbrough Pradhan,* BA, *and* R. P. Pohkrel,** FRCS

**Department of International Health, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD, USA*
***National Society for the Prevention of Blindness and World Health Organization Prevention of Blindness Programme, Kathmandu, Nepal*

Summary

Parental histories are often used to estimate the prevalence and the impact of interventions on child morbidity, but few studies have examined the agreement between parental histories and clinical examination. We compared clinical findings with a same-day parental morbidity history for pre-school-age children in rural Nepal. A 15 per cent sample of children from 40 wards in Sarlahi district, Nepal, was selected for participation and 814 same-day morbidity histories were obtained from parents. A clinician, masked to the parent's history, visited the household 2–4 h later and examined the child for signs of morbidity symptoms about which the parent had previously been questioned. Signs included measurement of temperature, respiratory rate, examination of stools, ear discharge, and presence of persistent cough. Agreement between the history and clinical examination was excellent for ear infection ($\kappa = 0.75$) and history of measles rash ($\kappa = 0.74$), moderate to poor for diarrhoea ($\kappa = 0.21$) and fever ($\kappa = 0.31$), and there was no evidence of agreement for dysentery ($\kappa = -0.01$), rapid breathing ($\kappa = 0.06$), and cough ($\kappa = 0.09$). The prevalence of dysentery, fever, cough, and rapid breathing was lower if clinical signs rather than histories were used. The prevalence of diarrhoea was higher if the presence of a loose stool in a cup rather than a history was used. The prevalence of ear infections and measles was comparable with both methods. The agreement between histories and clinical examination varies by morbidity type, as does the prevalence of morbidity estimated by one or other method.

Acknowledgements

This study was carried out under co-operative agreement no. DAN 0045-A-5094 between the Office of Nutrition, US Agency for International Development (USAID), Washington DC, the Center for Human Nutrition (CHN), and the Dana Center for Preventive Ophthalmology (DCPO) at Johns Hopkins University. It was a joint undertaking between CHN/DCPO and the National Society for the Prevention of Blindness, Kathmandu, Nepal.

This study was funded by USAID, with financial and technical assistance from Task Force Sight and Life (Roche, Basel), the United Nations Children's Fund (UNICEF), Nepal, and NIH grant no RR04060.

The Sarlahi Study Group comprises (in addition to the authors): Dr A. Sommer, D. N. Mandal, T. R. Sakya, N. Achariya, Dr J. M. Tielsch, Dr J. Humphrey, L. Clement, Dr J. Gmunder, Dr R. A. Adhikari, Dr F. R. Davidson, Dr D. Calder, and D. Piet.

The authors thank Dr Robert Black for his advice regarding morbidity assessment and methods for validation in a field setting.

Correspondence: Dr Joanne Katz, Johns Hopkins School of Hygiene and Public Health, Room 5515, 615 North Wolfe Street, Baltimore, MD 21205-2103, USA. Tel. 410 955 7016; Fax 410 550 6733.

Introduction

Many community-based studies use a parental history of morbidity symptoms to estimate the prevalence of common childhood illnesses or the impact of interventions on such illnesses. The advantages of these histories are that they can be completed rapidly by trained interviewers rather than by medically trained personnel who are more expensive and often in short supply in developing country settings. Since many population-based studies rely on morbidity histories, it is important to understand the accuracy of such histories in classifying children as ill or not, and in knowing whether histories provide a reasonable estimate of morbidity prevalence within a community compared to clinical examination. Several studies have validated parental histories by identifying children who present to hospitals or clinics with and without certain clinical diagnoses.[1–9] However, few studies have attempted to validate a history of symptoms obtained in a population-based setting against signs based on clinical examination.[10,11] We report here the results of a study to compare same-day parental morbidity histories for pre-school children against signs of illness identified on clinical examination in rural Nepal.

236

Methods

A randomized community trial to assess the impact of vitamin A supplementation on mortality, morbidity, and growth enrolled and followed approximately 38 000 preschool children every 4 months for a two-year period in the Sarlahi district of Nepal.[12-15] One year after the start of the trial, a 15 per cent systematic sample of children from each of 40 of the 261 wards in the community trial was selected to participate in a study to compare parental morbidity histories with a clinical examination of the children in their homes.

Parents of all children in the 40 wards were interviewed at the household by interviewers with 10th grade equivalency but without medical training, about the presence of a pre-specified list of morbidity symptoms in the past week. Parents were also asked about measles during the past 4 months. The 7-day morbidity history asked about the presence of diarrhoea (four or more loose, watery stools per day), dysentery (presence of blood in the stool independent of the response to the presence of diarrhoea), persistent cough, rapid breathing (breathing faster than normal and only asked if the child was reported to have a persistent cough), fever (high fever that was 'hot to the touch'), and ear discharge (discharge of pus from either ear).

If parents reported a symptom in the past 7 days, the interviewer also recorded whether the child had experienced the symptom on the day of the interview. At the time children had anthropometric measurements taken at a central site in the ward, parents of children in the 15 per cent sample were given a plastic cup for the collection of a stool sample which would be examined by the clinician visiting their house 2–4 h later. The results of the history were unknown to the clinician at the time of examination of the child and collection of the stool sample. Field work took place from September through to December, 1990.

Diarrhoea was considered to be present on the day of interview if the obtained stool conformed to the shape of the cup. If the stool did not conform to the shape of the cup, or if the cup did not contain any stool, then diarrhoea was considered absent. Dysentery was recorded as present if blood was visible within the stool sample, regardless of whether the stool conformed to the shape of the cup. Axillary temperatures were measured for a minimum of 2 min. Groin temperatures were taken in very small infants (less than 1 per cent of all children). Two 30 s respiratory rates were measured after the child had been observed to be sufficiently at rest for 3 min. A difference between the two counts of five or more necessitated further counts until the difference was no more than five. The two measurements were added to produce a respiratory rate per minute. Persistent cough was ascertained by noting whether the child coughed irregularly, persistently, or not at all, during the observation. The ears were examined for presence of pus in the external auditory canal using a flashlight. The clinician asked the parent about the presence of measles in the past 4 months. If the parent responded affirmatively, the clinician probed for more details about the rash and illness, and classified the response as a description consistent with a measles rash or not.

Agreement between clinical examination and same-day parental morbidity histories was based on the kappa (κ) statistic.[16] Different cut-off points were used for signs of illness, such as temperature above 37.5°C and above 38.5°C for comparison with a history of high fever. A respiratory rate of 60 breaths per minute or greater was used as the cut-off for the presence of rapid breathing. A respiratory rate cut-off of 50 or more for infants and 40 or more for 1–5-year-old children was also used. If the history of measles given to the clinician was consistent with a measles rash, then this was considered a positive history of measles on clinical examination as compared to a measles history given to lay interviewer.

After discussion with the communities, written consent was obtained from the village development committee leader for community participation. Individual consent was obtained verbally from parents. The study received ethical approval from the Joint Committee on Clinical Investigations of the Johns Hopkins Medical Institutions, and from the Nepal Health Research Council.

Results

A total of 5482 children resided in the 40 wards during the 1-year visit. A 4-month measles history was available for 5406 (98.6 per cent) children. A total of 4966 (90.6 per cent) had been under direct observation by the responding parent during the past week and had a 7-day morbidity history from the household visit (Fig. 1). The parents of 4525 children also provided same-day morbidity histories. A systematic sample of 850 children (15.5 per cent) was selected from the 5482 children for clinical examination. Of these, 815 (95.9 per cent) had been under direct observation by a parent during the past week and provided a 7-day morbidity history. A morbidity history on the day of interview was available for 814 of these children.

The same-day period prevalence of symptoms for the total and the clinical study groups were comparable, as was the 4-month measles history (Table 1). The prevalence of ear discharge based on clinical signs and the prevalence of measles based on history provided to the clinician were similar to those based on parental history. The prevalence of diarrhoea based on clinical signs was higher than that based on a same-day parental history. Dysentery, fever, cough, and rapid breathing all had lower prevalences based on clinical signs compared with parental histories.

The agreement between parental history and clinical examination was excellent for ear infection and for measles history (Table 2). Agreement was moderate to poor for diarrhoea and fever, and no agreement was

237

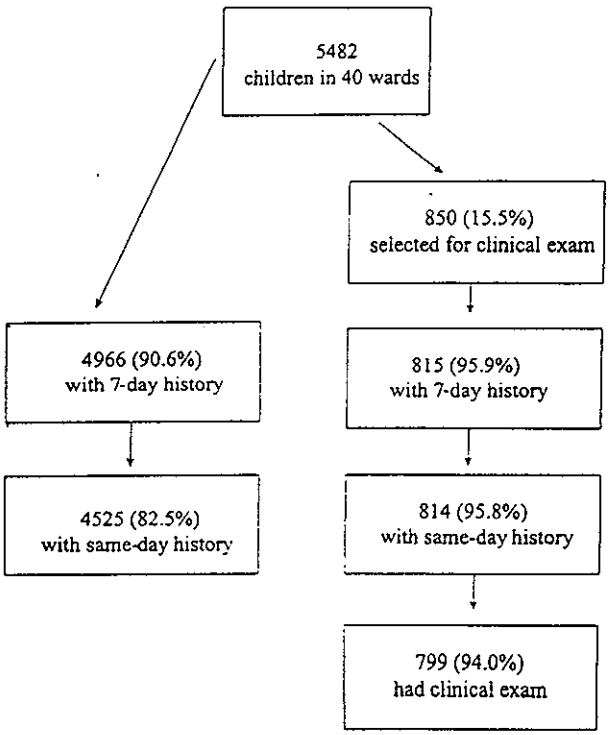


FIG. 1. Selection of children for clinical examination and number of children with parental histories and with clinical examination available for comparison of parental histories with clinical examinations.

seen for dysentery, persistent cough, and rapid breathing. Agreement for diarrhoea was better among children 1–5 years of age than among infants ($\kappa = 0.17$ vs. 0.03). Agreement was better for persistent cough among infants than among older children ($\kappa = 0.23$ vs. 0.07). Agreement was slightly better for ear infection among infants than older children ($\kappa = 0.88$ vs. 0.73). However, none of these differences was statistically significant.

Discussion

This study found variable agreement between parental

TABLE 1

Prevalence of morbidity symptoms by same-day history and 4-month measles history by clinical signs; Nepal, 1990

	Total cohort ($N = 4525$)		Clinical study ($N = 814$)		Clinically examined ($N = 799$)	
	n	%	*n*	%	*n*	%
Same-day history						
diarrhoea	130	2.9	17	2.1	36	4.6
dysentery	63	1.4	15	1.8	5	0.6
fever	128	2.8	21	2.6	5	0.6
cough	341	7.5	56	6.9	6	0.8
rapid breathing	130	2.9	21	2.6	6	0.8
ear discharge	248	5.5	52	6.4	53	6.6
4-month history	($N = 5406$)		($N = 814$)		($N = 813$)	
measles	224	4.1	44	5.4	38	4.7

There were no statistically significant differences in the prevalence of positive histories between the total sample and the examination sample.

238

TABLE 2
Agreement between same-day parental history of symptoms and signs on clinical examination; Nepal, 1990

Symptom	Clinical sign	κ value	95% confidence interval
Diarrhoea	Conforms to cup	0.21	0.11, 0.32
Dysentery	Blood visible in stool	−0.01	−0.02, 0.00
Fever	Temperature ≥38.5°	0.31	0.08, 0.55
	Temperature ≥37.8°	0.31	0.10, 0.53
Cough	Persistent on exam	0.09	−0.01, 0.18
Rapid breathing	Resp rate ≥60/min	0.06	−0.07, 0.20
	≥50/min for <1 year, ≥40/min for 1–5 years	0.02	−0.04, 0.07
Ear infection	Present on examination	0.75	0.65, 0.84
Measles	History of rash consistent	0.74	0.64, 0.85

history and clinical signs of illness. Ear discharge and a description of measles rash had excellent agreement, whereas same-day histories of rapid breathing, persistent cough, and dysentery did not agree with clinical signs on examination. The differences in agreement between different morbidities cannot be explained by selective absence of acutely ill children who might be away seeking medical care, since this is an area with extremely poor access to health care. The type of the disagreement also varied by morbidity. For diarrhoea, there were more children with a loose stool in the cup than reports by parents of one or more loose watery stool on the day of the interview. Parents reported more dysentery, fever, cough, and rapid breathing than were evident on clinical examination. These results were quite different from studies in which children were diagnosed through their attendance at hospitals or clinics.[1–9] In those studies, sensitivity was relatively high and specificity lower for diagnoses based on clinical examination and laboratory results. This is probably because children seeking care at hospitals or clinics are more severely ill, leading to higher sensitivity. Controls were sick children seeking care who did not meet specific definitions of disease, leading to a lower specificity. Lanata *et al.* used both clinic- and population-based controls and found that the specificity was higher among population-based controls for the diagnosis of pneumonia based on a history of fever (36 vs. 92 per cent) and rapid breathing (36 vs. 86 per cent).

Very few studies have reported a comparison of symptoms and specific clinical signs.[6,10] Gadomski *et al.* compared maternal histories of rapid breathing against respiratory rates at the time of admission to out-patient facilities and found sensitivities of 71 and 78 per cent and specificities of 61 and 48 per cent, depending on the local terms used for rapid breathing.[6] Black *et al.* validated a maternal history of abnormal stools against an assessment of stools collected in a cup from the mothers in a population-based setting similar to ours.[10] They found 80 per cent agreement between the history and the stool sample but did not report κ values or the sensitivity and specificity of this approach.

Since there was poor agreement between some of the signs and symptoms, one issue is whether the histories can be used to assess the prevalence of morbidity at a community level. The prevalence of morbidity based on a parental history was higher for dysentery, fever, cough, and rapid breathing than for prevalence based on clinical signs. It was comparable for ear discharge and measles, and lower for diarrhoea. Hence, the bias in the use of histories for community assessment of morbidity appears to be symptom specific.

Very few studies have attempted to compare parental histories with clinical examination in a population-based setting. In spite of the problems concerning whether the clinical examination really can 'validate' the parental histories, these data provide scarce information about the accuracy of such histories in rural South Asia. The accuracy of parental histories varies substantially from one morbidity to another. This has implications for the use of these histories in estimating morbidity prevalence and in estimating the impact of treatment on different morbidities.

References

1. Schulpen TWJ, Swinkels WJ. Machakos project studies: agents affecting health of mother and child in a rural area of Kenya – XIX: the utilization of health services in a rural area of Kenya. Trop Geog Med 1980; 32: 340–9.
2. Alonso PL, Bowman A, Marsh K, Greenwood BM. The accuracy of the clinical histories given by mothers of seriously ill African children. Ann Trop Ped 1987; 7: 187–9.
3. Greenwood BM, Bradley AK, Greenwood AM, *et al.* Mortality and morbidity from malaria among children in a rural area of the Gambia, West Africa. Trans R Soc Trop Med Hyg 1987; 81: 478–86.
4. Kalter HD, Gray RH, Black RE, Gultiano SA. Validation of the diagnosis of childhood morbidity using maternal health interviews. Int J Epidemiol 1991; 20: 193–8.
5. Cherial T, John TJ, Simoes E, Steinhoff MC, John M. Evaluation of simple clinical signs for the diagnosis of acute lower respiratory tract infection. Lancet 1988; ii: 125–8.
6. Gadomski AM, Aref GH, Hassanien F, *et al.* Caretaker recognition of respiratory signs in children: correlation with physical examination findings, X-ray diagnosis and pulse oximetry. Int J Epidemiol 1993; 22: 1166–73.
7. Harrison LH, Moursi S, Guinena AH, *et al.* Maternal

239

reporting of acute respiratory infection in Egypt. Int J Epidemiol 1995; 24: 1058–63.
8. Lanata CF, Quintanilla N, Verastegui HA. Validity of a respiratory questionnaire to identify pneumonia in children in Lima, Peru. Int J Epidemiol 1994; 23: 827–34.
9. Watkins CJ, Burton P, Leeder S, Sittampalam Y, Wever AMJ, Wiggins R. Doctor diagnosis and maternal recall of lower respiratory illness. Int J Epidemiol 1982; 11: 62–6.
10. Black RE, Brown KH, Becker S, Yunus M. Longitudinal studies of infectious disease and physical growth of children in rural Bangladesh. Am J Epidemiol 1982; 115: 305–14.
11. Belcher DW, Neumann AK, Wurapa FK, Lourie IM. Comparison of morbidity interviews with a health examination survey in rural Africa. Am J Trop Med Hyg 1976; 75: 751–8.
12. West KP, Pokhrel RP, Katz J, *et al*. Efficacy of vitamin A in reducing preschool child mortality: a randomized double-masked community trial in Nepal. Lancet 1991;338:67–71.
13. Pradhan EK, Katz J, LeClerq SC, West KP. Data management for large community trials in Nepal. Controlled Clin Trials 1994; 15: 220–43.
14. West KP, Katz J, Shrestha SR, *et al*. Mortality of infants under six months of age supplemented with vitamin A: a randomized double-masked community trial. Am J Clin Nutr 1995; 62: 143–8.
15. West KP, Khatry SK, LeClerq SC, *et al*. Tolerance of young infants to a single, large dose of vitamin A: a randomized community trial in Nepal. Bull WHO 1992; 70: 733–9.
16. Fleiss JL. Statistical Methods for Rates and Proportions, 2nd edn. Wiley, New York, 1981; 211–34.

240

J. Parasitol., 84(3), 1998 p. 647–651
© American Society of Parasitologists 1998

Ancylostoma duodenale Is Responsible for Hookworm Infections among Pregnant Women in the Rural Plains of Nepal

R. C. Navitsky, M. L. Dreyfuss, J. Shrestha†, S. K. Khatry†, R. J. Stoltzfus*, and Albonico‡, Center for Human Nutrition, School of Hygiene and Public Health, Johns Hopkins University, Baltimore Maryland 21205-2103, †National Society for the Prevention of Blindness, Kathmandu, Nepal, and ‡Schistosomiasis and Intestinal Parasites Unit, Division of Control of Tropical Diseases, World Health Organization, Geneva, Switzerland; *corresponding author

ABSTRACT: Fecal specimens from 292 pregnant women (ages 15–40 yr) and 129 infants (ages 10–20 wk) were examined for helminth eggs by the Kato–Katz method and cultured for helminth larvae identification using a modified Harada–Mori method. These specimens were collected from June 1995 through July 1996 in Sarlahi District in the southern rural plains of Nepal. Among pregnant women, the prevalence of helminth infection by the Kato–Katz method was 78.8%, 56.2%, and 7.9% for hookworm, *Ascaris lumbricoides*, and *Trichuris trichiura*, respectively. Using the modified Harada–Mori method, 66.1% and 2.0% of women's fecal cultures were positive for hookworm and *Strongyloides stercoralis*, respectively. All of the cultured hookworm larvae were identified as *Ancylostoma duodenale*. Among infants, 1 specimen was

241

positive for hookworm and 1 for *A. lumbricoides* using the Kato–Katz method. The modified Harada–Mori method detected no larvae in specimens from infants. There was 81.8% agreement between the 2 methods for the detection of hookworm infection. *Ancylostoma duodenale* is endemic in this study population and highly prevalent in pregnant women.

Hookworms infect over a billion people worldwide, with prevalences as high as 95% in certain communities of developing countries (Hoagland and Schad, 1978; Walsh and Warren, 1979). In Nepal, intestinal helminth infection is a contributor to malnutrition and anemia (National Planning Commission, 1992a). Hookworm infection contributes to iron-deficiency anemia in populations with poor diets (Stoltzfus et al., 1997), and heavy infections cause severe anemia and retard the growth and development of children (Hotez and Pritchard, 1995). It has also been cited as a major concern for women's health because of its potential impact on maternal anemia and reproductive outcomes (World Health Organization, 1994a).

Iron-deficiency anemia is the most well documented consequence of hookworm infection that causes chronic intestinal blood loss (Roche and Layrisse, 1966). Women and children usually have the lowest iron stores, making them most susceptible to developing iron-deficiency anemia from hookworm infection (Layrisse and Roche, 1964). This is particularly critical in pregnant women, due to the increased iron demand of pregnancy.

The 2 major species of hookworm that infect humans are *Ancylostoma duodenale* and *Necator americanus*. Their pathogenicity, mode of transmission, geographic distribution, and biology are different and may influence the morbidity of hookworm disease. In general, *A. duodenale* is found at higher elevations, in northern regions, and in more extreme climates, whereas *N. americanus* is found in lowlands and in more tropical areas, though both species can be endemic to the same region (Hoagland and Schad, 1978). *Ancylostoma duodenale* is more pathogenic (Hoagland and Schad, 1978), causing about 4–5 times greater blood loss and releasing twice the number of eggs per female as *N. americanus* (Pawlowski et al., 1991). Furthermore, it has been suggested that *A. duodenale* can be vertically transmitted from mother to infant (Schad, 1991).

The objective of the present study was to determine the species of hookworm and other helminths infecting pregnant women and infants in the Terai region of Nepal. This study was in the context of a larger research program aimed at developing interventions to improve women's reproductive health in rural south Asia.

The study area consisted of 27 wards in Sarlahi District in the southern, rural plains of Nepal. This area is at an elevation of approximately 80 m above sea level on the Gangetic flood plain at a latitude of approximately 27° N. There is a wet monsoon season from June through September, with a cool season followed by a hot dry season for the rest of the year. Temperatures are hottest in June (1990 average maximum temperature: 34.0 C) and coldest in January (1990 average maximum temperature: 22.7 C; National Planning Commission, 1993). Annual rainfall was 136.4 cm during 1990 (National Planning Commission, 1993). Latrines are not widely available in this area. The women and infants whose data are presented here were participating in a substudy of the Nepal Nutrition Intervention Project–Sarlahi (NNIPS). Data were collected at the NNIPS clinic in Hariaun, Sarlahi District, Nepal from June 1995 to July 1996.

Pregnant women in the study communities were identified by weekly household interview, which ascertained menstrual status and women's self-report of pregnancy. Pregnant women were invited to come to the clinic for a health and nutrition examination. At 3-mo postpartum, the mothers were asked to return to the clinic with their infants for a follow-up examination. Helminth infections were identified from stool specimens of women at the pregnancy visit and of infants at the follow-up examination. One day before the women or infants were to present to the clinic, they were given clean plastic containers and instructed to collect the stool specimens that evening or the next morning. The following day, they were driven by vehicle to the clinic and their fecal specimens were collected and processed. Approximately 70% of pregnant women and 57% of infants returned with a fecal specimen.

These specimens were analyzed by the Kato–Katz method (World Health Organization, 1994b) to identify and quantify hookworm, *Ascaris lumbricoides*, and *Trichurus trichiura* eggs. Women found to have severe anemia were treated with oral iron and anthelminthics. Otherwise, anthelminthics were given postpartum. To determine the hookworm species, each specimen was also cultured using the Harada–Mori method (Harada and Mori, 1955), modified according to Garcia and Bruckner (1993) to increase the efficacy of the culture method. Approximately 1–2 g of feces were mixed with the same amount of activated granulated charcoal into a homogenous paste. This paste was spread onto filter paper that had been folded around 2 microscope slides and placed in a Petri dish. Six to 10 ml of boiled and filtered water was added carefully to the petri dish. These cultures were covered and labeled and then incubated at room temperature (20–37 C). The cultures were incubated for 7–10 days and the culture water collected in test tubes. The tubes were heated in boiling water for 5 min to kill the infective larvae. After cooling, 3–5 drops of 10% formalin were added to the tubes to preserve the specimen for transport to the U.S.A. Two to 3 drops of the specimen sediment were viewed microscopically at 10× and 40× total magnification. The identity and quantity of *A. duodenale, N. americanus,* and *Strongyloides stercoralis* species larvae were determined using the criteria given in Pawlowski et al. (1991).

Results are reported on 421 (292 maternal and 129 infant) of the 429 specimens cultured because 8 of the specimen test tubes were damaged in transit. The mean age of the pregnant women was 24 yr (range 15–40 yr), and mean gestational age at the time of the clinic visit was 19 wk. Pregnancy status was verified by a β-human chorionic gonadotropin urine test. The mean age of the infants was 12 wk (range 10–20 wk).

The prevalence of hookworm infection among pregnant women was 78.8% using the Kato–Katz method (Table I). *Ascaris lumbricoides* and *T. trichiura* eggs were also found in the specimens of 56.2% and 7.9% of the women, respectively. Using the modified Harada–Mori fecal culture method, the prevalence of hookworm infection was 66.1% among pregnant women. All of the cultured larvae were identified as *A. duodenale* (100%). *Strongyloides stercoralis* larvae were found in 2.0% of the women's fecal cultures.

Among infants, only 1 (0.8%) specimen was positive for hookworm and 1 (0.8%) for *A. lumbricoides* by the Kato–Katz

242

TABLE I. Helminth infection in Nepalese pregnant women and their infants.

Species	Prevalence: Pregnant women % (n)	Prevalence: Infants % (n)
Determined by Kato–Katz method:		
Hookworm*	78.8 (230)	0.8 (1)
Ascaris lumbricoides	56.2 (164)	0.8 (1)
Trichuris trichiura	7.9 (23)	0
Determined by Harada–Mori method:		
Hookworm		
Ancylostoma duodenale	66.1 (193)	0
Necatur americanus	0	0
Strongyloides stercoralis	2.0 (6)	0

* Hookworm species are indistinguishable by this method.

TABLE II. Comparison of Kato–Katz and modified Harada–Mori methods for the diagnosis of hookworm infection in Nepalese pregnant women.

Kato–Katz results	Modified Harada–Mori fecal culture result: Negative n (%)	Positive for *Ancylostoma duodenale* n (%)	Positive for *Necatur americanus* n (%)	All n (%)
Negative	54	8	0	62 (21.2)
Positive	45	185	0	230 (78.8)
All	99 (33.9)	193 (66.1)	0	292

method (Table I). None of the infant specimens was infected with *T. trichiura*. When infant fecal specimens were cultured, no hookworm or other helminth larvae were found.

A typical distribution of hookworm fecal egg counts was found, with the majority of the infected women having light infections (Fig. 1). Moderate to severe infection (≥2,000 eggs per g feces) was present in 54 (18.5%) of the women.

Neither the overall prevalence of hookworm infection nor the intensity of infection differed significantly between seasons. Hookworm prevalence in the monsoon season (June–September) was 83.5% and in the remaining dry months of the year was 77.5% ($P = 0.23$ by Student's t-test). The geometric mean hookworm burden was 842 eggs per g (epg) feces during the monsoon season and 614 epg during other times of the year ($P = 0.09$ by Student's t-test).

The agreement between the Kato–Katz and the modified Harada–Mori fecal culture methods for diagnosing hookworm infection was 81.8% for the women's fecal specimens (Table II). Using the Kato–Katz method as a standard, the modified Harada–Mori method in the pregnant women's specimens was 80.4% sensitive, 87.1% specific, and had a positive predictive value of 95.8%. However, the primary purpose of the Harada–

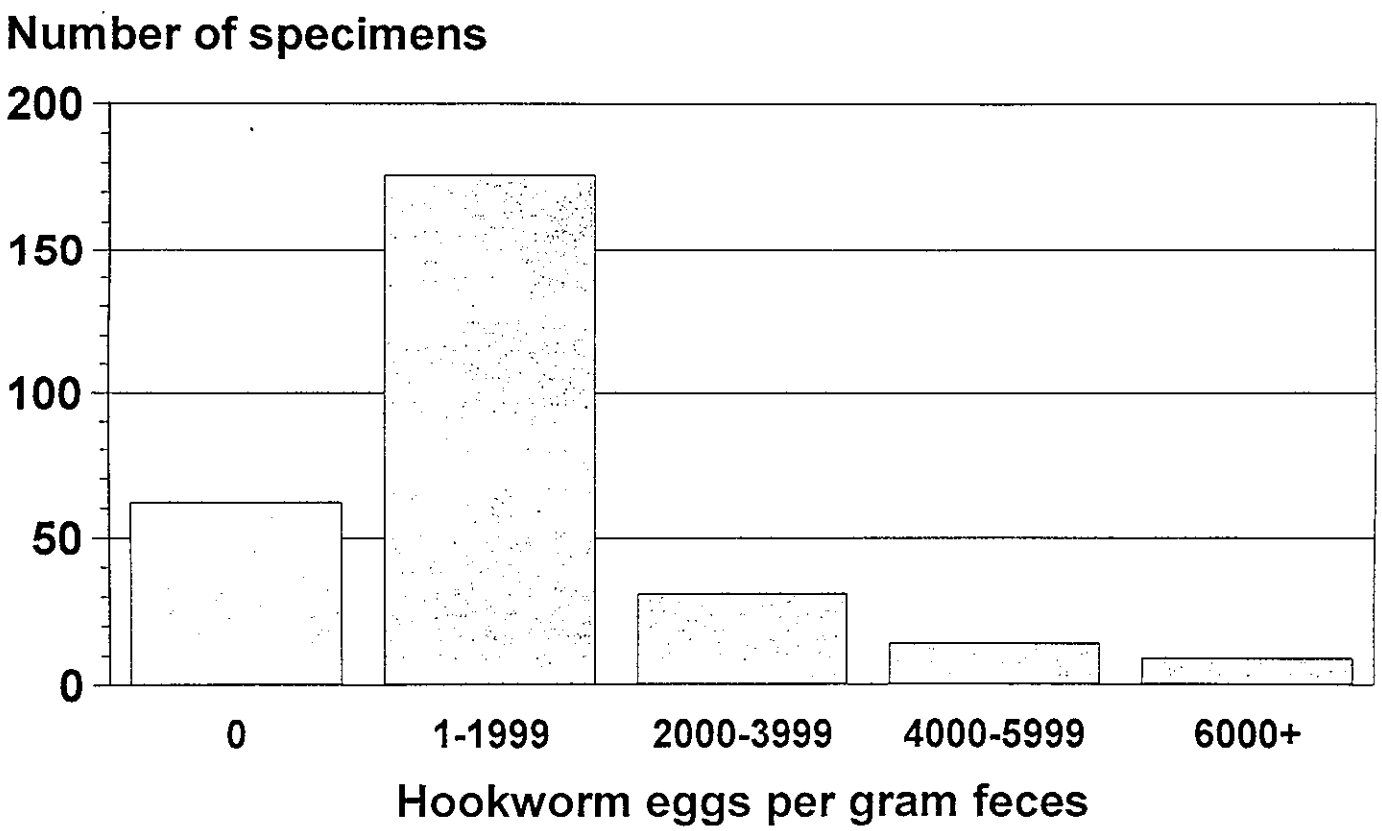


FIGURE 1. Distribution of hookworm infection intensity in pregnant women determined by the Kato–Katz method (n = 292).

243

Mori test is to distinguish the species of hookworm, not to diagnose infection.

These findings demonstrate that hookworm infection is endemic among pregnant women in Sarlahi District and likely in other ecologically similar regions in Nepal and northern India. Hookworm infection is recognized as an important contributor to iron-deficiency anemia among adolescent girls and women of child-bearing age in many developing countries where it is endemic (World Health Organization, 1994a).

This study is the first report in the peer-reviewed literature identifying *A. duodenale* in Nepal. The presence of only 1 species of hookworm, *A. duodenale,* among this study population is consistent with previous findings that this species is found in the more northern regions of the tropics (Darling, 1912) and is more common in the northern Indian subcontinent (Hoagland and Schad, 1978). However, Nawalinski et al. (1978) found *N. americanus* to be a significant proportion of hookworm infection in rural communities in India, approximately 500 km southeast of our study area. The higher hookworm egg counts observed during the monsoon season in our study are also consistent with other studies in south Asia (Nawalinski et al., 1978).

Given the significant blood loss associated with *A. duodenale* infection, our findings suggest that hookworm infection may be a major cause of anemia and poor reproductive health in this region of Nepal. This is currently under investigation. It is noteworthy that Nepal has a high maternal mortality rate (850 per 100,000 live births; National Planning Commission, 1992b), and maternal mortality is frequently associated with anemia (Ross and Thomas, 1996).

The modified Harada–Mori method provided a simple method by which to culture larvae for species identification. This method has also been successfully used in Pemba, Tanzania by Albonico et al. (1998), where they were able to identify larvae from both species. As in the present study, the modified Harada–Mori method was found to be less sensitive than the Kato–Katz method for the identification of hookworm in the Tanzania study.

There was no evidence of vertical transmission of hookworm from pregnant women to their infants in this study sample, with the possible exception of a single infant specimen positive for hookworm by the Kato–Katz method. However, infant stool specimens were almost always watery, such that the Kato–Katz method was difficult to conduct and the Harada–Mori method may have been less sensitive. Nawalinski et al. (1978) found that if the consistency of the specimens was more diarrheic, then the number of epg of feces was reduced by as much as a third. Therefore, these methods may not be sensitive enough to detect possible light infections in infants, and the absence of hookworm larvae in the infant specimens by the Harada–Mori culture method does not rule out the possibility of vertical transmission. A more sensitive technique of hookworm detection might identify the presence of light infections in infants. Methods such as the formol–ether concentration technique (World Health Organization, 1994b), the zinc sulfate centrifugal floatation technique (Neva and Brown, 1994), or perhaps polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technology as developed by Hawdon (1996) may be sufficiently sensitive to find infant infection if it exists.

This study was carried out jointly by the National Society for the Prevention of Blindness (Nepal Netra Jyoti Sangh), Kathmandu, Nepal and the Center for Human Nutrition, Johns Hopkins University, Baltimore, Maryland, under cooperative agreement no. DAN-0045-A-00-5094-00 with the Office of Health and Nutrition, U.S. Agency for International Development (USAID), Washington, D.C. Both technical and financial support were also provided by the Schistosomiasis and Intestinal Parasites Unit, Division of Control of Tropical Diseases, World Health Organization, Geneva, Switzerland. The authors thank Lorenzo Savioli, Director of this unit, whose guidance and support in the design and conduct of this study were crucial to its success. The authors also thank G. A. Schad, University of Pennsylvania, and T. Nawalinski for providing assistance in the training for identification of the specimens, as well as D. I. Pritchard, University of Nottingham, for providing pure specimens of *N. americanus* larvae for comparison. Also, the authors acknowledge the hard work and dedication of our colleagues at the NNIPS project, especially Kalawati Giri and Rajeshwari Kafle.

LITERATURE CITED

ALBONICO, M., R. J. STOLTZFUS, L. SAVIOLI, J. TIELSCH, H. M. CHWAYA, E. ERCOLE, AND G. CANCRINI. 1998. Epidemiologic evidence for a differential effect of hookworm species, *Ancylostoma duodenale* and *Necator americanus,* on iron status of children. International Journal of Epidemiology **27:** 530–537.

CHOWDHURY, A. B., G. A. SCHAD, AND E. L. SCHILLER. 1968. The prevalence of intestinal helminths in religious groups of a rural community near Calcutta. American Journal of Epidemiology **87:** 313–317.

DARLING, S. T. 1912. Observations on the geographical and ethnological distribution of hookworms. Parasitology **12:** 217–233.

GARCIA, L. S., AND D. A. BRUCKNER. 1993. Diagnostic medical parasitology, 2nd ed. American Society for Microbiology, Washington D.C., 555 p.

HARADA, Y., AND O. MORI. 1955. A new method for culturing hookworm. Yonago Acta Medica **1:** 177–195.

HAWDON, J. M. 1996. Differentiation between the human hookworms *Ancylostoma duodenale* and *Necator americanus* using PCR-RFLP. Journal of Parasitology **82:** 642–647.

HOAGLAND, K. E., AND G. A. SCHAD. 1978. *Necator americanus* and *Ancylostoma duodenale*: Life history parameters and epidemiological implications of the two sympatric hookworms of humans. Experimental Parasitology **44:** 36–49.

HOTEZ, P. J., AND D. I. PRITCHARD. 1995. Hookworm infection. Scientific American **272:** 68–74.

LAYRISSE M., AND M. ROCHE. 1964. The relationship between anemia and hookworm infection: Results of surveys of rural Venezuelan population. American Journal of Hygiene **79:** 279–301.

NATIONAL PLANNING COMMISSION, H. M. G. 1992a. Children and women of Nepal, a situation analysis. UNICEF, Kathmandu, Nepal, p. 68–76.

———. 1992b. The eighth plan (1992–1997). His Majesty's Govenment, Kathmandu, Nepal.

———. 1993. Statistical yearbook of Nepal. Central Bureau of Statistics, Kathmandu, Nepal.

NAWALINSKI, T., G. A. SCHAD, AND A. B. CHOWDHURY. 1978. Population biology of hookworms in children in rural West Bengal I and II. American Journal of Tropical Medicine and Hygiene **27:** 1152–1173.

NEVA, F. A., AND H. W. BROWN. 1994. Basic clinical parasitology, 6th ed. Appleton and Lange, Norwalk, Connecticut, 338 p.

PAWLOWSKI, Z. S., G. A. SCHAD, AND G. J. SCOTT. 1991. Hookworm infection and anaemia. Approaches to prevention and control. World Health Organization, Geneva, Switzerland, 26 p.

ROCHE, M., AND M. LAYRISSE. 1966. The nature and cause of "hookworm anemia." American Journal of Tropical Medicine and Hygiene **15:** 1031–1100.

244

ROSS, J. S., AND E. L. THOMAS. 1996. Iron-deficiency anemia and maternal mortality, PROFILES 3 Working Notes Series No. 3, Washington, D.C., p. 1–15.

SCHAD, G. A. 1991. Presidential address—Hooked on hookworms. Journal of Parasitology **77:** 177–186.

STOLTZFUS, R. J., H. M. CHWAYA, J. M. TIELSCH, K. J. SCHULZE, M. ALBONICO, AND L. SAVIOLI. 1997. Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: The importance of hookworms. American Journal of Clinical Nutrition **65:** 153–159.

WALSH, J. A., AND K. S. WARREN. 1979. Selective primary health care: An interim strategy for disease control in developing countries. New England Journal of Medicine **301:** 967–974.

WORLD HEALTH ORGANIZATION. 1994a. Report of the W.H.O. informal consultation on hookworm infection and anemia in girls and women. World Health Organization, Geneva, Switzerland. WHO/CTD/SIP/96.1.

———. 1994b. Bench aids for the diagnosis of intestinal parasites. World Health Organization, Geneva, Switzerland.

245

CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, July 1998, p. 583–587 Vol. 5, No. 4
1071-412X/98/$04.00+0
Copyright © 1998, American Society for Microbiology. All Rights Reserved.

Expression of Adhesion Molecules and CD28 on T Lymphocytes during Human Immunodeficiency Virus Infection

SUK W. PARK,[1] WALTER ROYAL III,[1] RICHARD D. SEMBA,[2]* GORDON W. WIEGAND,[3] AND DIANE E. GRIFFIN[1,4]

Departments of Neurology,[1] Ophthalmology,[2] and Medicine,[3] School of Medicine, and Department of Molecular Microbiology and Immunology,[4] School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland

Received 7 April 1997/Returned for modification 6 February 1998/Accepted 3 April 1998

Adhesion molecules, which play a major role in lymphocyte circulation, have not been well characterized in human immunodeficiency virus (HIV) infection. T-lymphocyte populations, including CD3, CD4, CD28, and adhesion molecules (L selectin, LFA-1, VLA-4, and ICAM-1) were measured by flow cytometry in a cross-sectional study of 100 HIV-infected and 49 HIV-seronegative adults. HIV-infected adults had lower numbers of $CD3^+$ lymphocytes expressing L selectin ($P < 0.0001$) and VLA-4 ($P < 0.01$) and higher numbers of $CD3^+$ lymphocytes expressing LFA-1^{bright} ($P < 0.002$) than did HIV-negative adults. By $CD4^+$-lymphocyte count category (>500, 200 to 500, or <200 cells/μl), HIV-infected adults with more advanced disease had lower percentages of $CD3^+$ lymphocytes expressing L selectin and VLA-4 and higher percentages of $CD3^+$ lymphocytes expressing LFA-1. The percentages of $CD3^+$ $CD28^+$ lymphocytes and of $CD3^+$ L $selectin^+$ lymphocytes were positively correlated (Spearman coefficient = 0.86; $P < 0.0001$), and the percentage of $CD3^+$ $CD28^+$ lymphocytes and the $CD3^+$ LFA-1^{bright} lymphocyte/$CD3^+$ LFA-1^{dim} lymphocyte ratio were negatively correlated (Spearman coefficient = −0.92; $P < 0.00001$). The results of this study suggest that HIV infection is associated with altered expression of adhesion molecules.

Human immunodeficiency virus (HIV) infection is characterized by progressive loss of $CD4^+$ lymphocytes, increase in $CD8^+$ lymphocytes, and major functional abnormalities in lymphocyte function (7). Prior to the development of AIDS there is homeostasis of $CD3^+$ lymphocytes, in which $CD8^+$ lymphocytes increase in number to compensate for the loss of $CD4^+$ lymphocytes (16). The later progression of HIV disease is associated with major changes in T-lymphocyte populations, including loss of CD28 surface antigen from both $CD4^+$ and $CD8^+$ lymphocytes (3, 5).

Lymphocyte adhesion molecules include the selectin, integrin, and immunoglobulin gene superfamilies (13, 22). L selectin (CD62L), which plays a major role in lymphocyte recirculation to peripheral lymph nodes, is normally expressed on approximately 70 to 80% of T lymphocytes (23). Lymphocyte function-associated antigen 1 (LFA-1), an integrin which is expressed on virtually all peripheral blood leukocytes, including T lymphocytes, is involved in lymphocyte-endothelial cell adhesion (13). The ligands for LFA-1 are two members of the immunoglobulin gene superfamily, intercellular adhesion molecules 1 (ICAM-1) and -2 (ICAM-2). Very late antigen 4 (VLA-4) is expressed on T cells and is involved in lymphocyte adhesion to endothelial cells. The expression of these adhesion molecules during HIV infection is not well understood. We conducted a clinic-based, cross-sectional study of major T-lymphocyte populations and adhesion molecules in HIV-infected adults.

MATERIALS AND METHODS

The study population consisted of a consecutive sample of HIV-seropositive and HIV-seronegative adults seen in the outpatient clinics at the Johns Hopkins Hospital, Baltimore, Md. The study design was cross-sectional. After written informed consent was obtained, blood samples were collected by venipuncture. All lymphocyte populations were studied from a single blood sample drawn from each subject. Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood with Ficoll-Hypaque (Pharmacia, Piscataway, N.J.), washed in Hanks' buffered salt solution, and resuspended in phosphate-buffered saline with 1% fetal bovine serum and 0.1% sodium azide (assay buffer). Conjugated monoclonal antibodies to human CD3, CD4, CD14, CD28, CD45, L selectin (Becton Dickinson Immunocytometry Systems, San Jose, Calif.), LFA-1, ICAM-1 (AMAC, Westbrook, Maine), and VLA-4 (Endogen, Boston, Mass.) were used in two-color (fluorescein isothiocyanate and phycoerythrin) analyses.

PBMC were prepared according to the Centers for Disease Control and Prevention guidelines for leukocyte immunophenotyping in HIV infection (4). One million PBMC were placed in 1.5-ml microcentrifuge tubes, pelleted, and resuspended in 50 μl of assay buffer. The PBMC were stained with combinations of monoclonal antibodies for 20 min at 4°C. All samples were kept at 4°C and in 1% sodium azide throughout the staining process to minimize the likelihood of cell activation. The PBMC were washed twice with 1 ml of assay buffer, fixed with 1% paraformaldehyde in phosphate-buffered saline, and analyzed by direct two-color immunofluorescence. Isotype-matched monoclonal antibodies from the same commercial suppliers served as controls for fluorescence marker settings and identification of nonspecific staining.

Flow cytometry was performed by using two-color (FACStar Plus; Becton Dickinson) analysis. Fluorescent microbeads (Simply Cellular and Quantum Simply Cellular beads; Flow Cytometry Standards Corporation, Research Triangle Park, N.C.) were used to calibrate the flow cytometer daily and to control for reproducibility of the assay. For each sample, 20,000 ungated events were collected. The lymphocyte population was selected by backgating for $CD45^{bright}$ $CD14^-$ cells from the profile of the sample stained with fluorescein isothiocyanate anti-CD45–phycoerythrin anti-CD14 such that >95% of the forward-scatter versus side-scatter lymphocyte gate was $CD45^{bright}$ and $CD14^-$. Thus, >95% of the lymphocyte gate contained lymphocytes. Quadrant analysis cursors were set from each individual and adjusted from the isotype control sample so that >98% of the gated cells were double negative. Dot plots, histograms, and raw statistical information were generated from this gated lymphocyte population for each analyzed sample. Any shift in population and the presence or absence of dim cells versus bright cells were noted. The raw subset percentage value was divided by the $CD45^{bright}$ $CD14^-$-lymphocyte percentage to correct for nonlymphocyte events in the lymphocyte gate. The $CD4^+$-lymphocyte count was calculated by multiplying the absolute lymphocyte count by the corrected $CD4^+$-lymphocyte percentage from flow cytometric analysis. The absolute lymphocyte count was measured by an automated cell counter (Coulter Diagnostics, Hialeah, Fla.) in the clinical laboratory of Johns Hopkins Hospital. In subjects with large unclassified cells, the lymphocyte count was adjusted after a manual count of a blood

* Corresponding author. Mailing address: Ocular Immunology Service, Suite 700, 550 North Broadway, Baltimore, MD 21205. Phone: (410) 955-3572. Fax: (410) 955-0629. E-mail: rdsemba@welchlink.welch.jhu.edu.

246

TABLE 1. Expression of adhesion molecules on $CD3^+$ lymphocytes

Marker	$CD3^+$ lymphocytes expressing marker [median (25th-75th percentile)]									
	HIV-seronegative adults					HIV-seropositive adults				
	n^a	No. (cells/μl)	*P* value	%	*P* value	n^a	No. (cells/μl)	*P* value	%	*P* value
L selectin^{high}	49	1,242 (1,104-1,412)	0.0001	83.1 (78.1-87.2)	0.0001	100	513 (219-808)	0.0001	56.1 (47.9-66.7)	0.0001
L selectin^{low}		246 (186-339)	0.007				361 (202-596)	0.007		
VLA-4$^+$	22	1,501 (1,371-1,659)	0.01	98.6 (97.8-99.0)	0.0001	23	914 (620-1,130)	0.01	93.0 (80.8-96.5)	0.0001
VLA-4$^-$		22 (16-33)	0.005				64 (51-88)	0.005		
LFA-1^{bright}	49	382 (239-538)	0.002	27.6 (15.5-35.3)	0.0001	100	616 (296-938)	0.002	65.1 (55.6-77.1)	0.0001
LFA-1^{dim}		1,097 (891-1,283)	0.0001				248 (131-472)	0.0001		
ICAM-1$^+$	13	547 (336-739)	NS^b	38.3 (25.9-48.7)	0.05	35	532 (191-708)	NS	48.7 (37.4-55.3)	0.05
ICAM-1$^-$		882 (732-963)	0.004				491 (296-729)	0.004		

^{a} *n*, number (*n*) of patients.
^{b} NS, not significant.

smear. Large unclassified cells were included in the calculation of total lymphocytes.

Comparisons of nonparametric data were made between HIV-seronegative and HIV-seropositive individuals by the Mann-Whitney *U* test. Within the HIV-seropositive group, individuals were divided into strata based upon the number of $CD4^+$ lymphocytes (>500, 200 to 500, and <200 cells/μl). Lymphocyte populations were compared within these three groups by the Kruskal-Wallis test. Analysis of variance was used to examine differences between groups, adjusted by age and sex. The level of statistical significance used in this study was a *P* value of <0.05. Median and interquartile ranges were used to describe lymphocyte population distributions, since the distributions were asymmetric for most populations.

RESULTS

Expression of adhesion molecules on $CD3^+$ lymphocytes. The percentages and absolute counts of $CD3^+$ lymphocytes expressing L selectin, VLA-4, LFA-1, and ICAM-1 are shown in Table 1. HIV-seropositive individuals had significantly lower percentages and absolute counts of $CD3^+$ lymphocytes expressing L selectin and VLA-4 than did HIV-seronegative individuals. The percentages of $CD3^+$ lymphocytes expressing LFA-1^{bright} and ICAM-1 were significantly higher in HIV-seropositive individuals. There was no significant difference in the absolute numbers of $CD3^+$ lymphocytes expressing ICAM-1 between HIV-seropositive and HIV-seronegative individuals; however, HIV-seronegative individuals had significantly higher absolute numbers of $CD3^+$ ICAM-1$^-$ lymphocytes than did HIV-seropositive individuals. Representative immunofluorescence histograms of adhesion molecules on $CD3^+$ lymphocytes are shown in Fig. 1.

The expression of adhesion molecules on $CD3^+$ lymphocytes was examined according to the $CD4^+$-lymphocyte count (>500, 200 to 500, or <200 cells/μl) among HIV-seropositive individuals (Fig. 2). Lower proportions of $CD3^+$ lymphocytes expressing L selectin were found in HIV-seropositive individuals with lower $CD4^+$ lymphocyte counts. The proportion of $CD3^+$ lymphocytes expressing VLA-4 tended to decrease slightly among HIV-seropositive individuals with lower $CD4^+$ counts. With decreasing $CD4^+$-lymphocyte counts among HIV-seropositive adults, the proportions of $CD3^+$ lymphocytes expressing LFA-1 increased.

Expression of $CD28^+$ on T-lymphocyte subsets. The percentages and absolute counts of $CD3^+$, $CD4^+$, and $CD8^+$ lymphocytes expressing $CD28^+$ are shown in Table 2. HIV-seropositive individuals had significantly lower absolute counts and percentages of $CD3^+$, $CD4^+$, and $CD8^+$ lymphocytes expressing $CD28^+$ than did HIV-seronegative individuals. Expression of $CD28^+$ on $CD3^+$, $CD4^+$, and $CD8^+$ lymphocytes was examined according to the $CD4^+$-lymphocyte count (>500, 200 to 500, or <200 cells/μl) among HIV-seropositive individuals (Fig. 3). Lower proportions of $CD3^+$ lymphocytes expressing $CD28^+$ were found in HIV-seropositive individuals

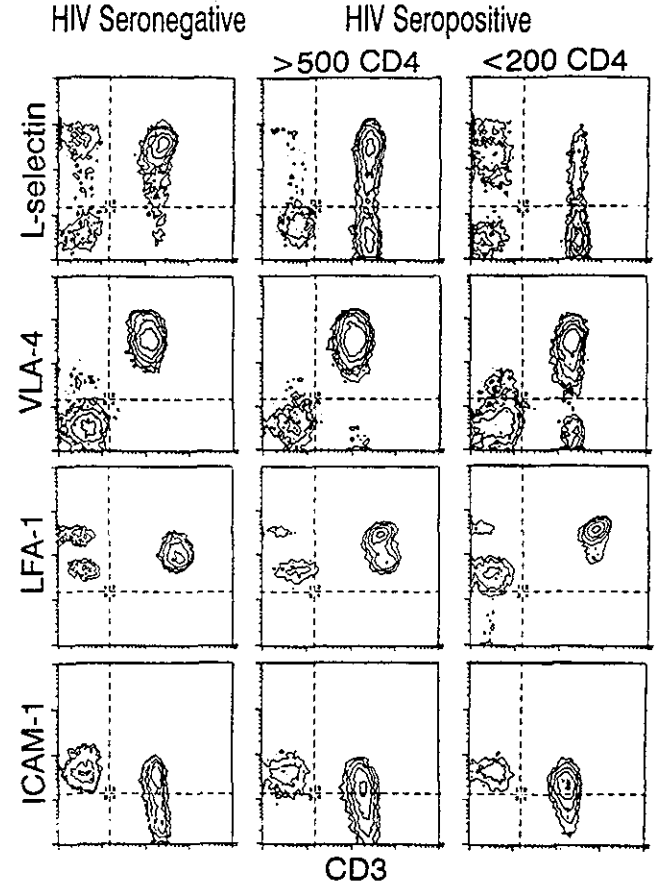


FIG. 1. Representative immunofluorescence histograms of adhesion molecules and CD3 expression on lymphocytes from HIV-seronegative and HIV-seropositive individuals. Quadrant settings for the four regions were established such that <2% of positive events in the isotype control sample fell in any positive region.

247

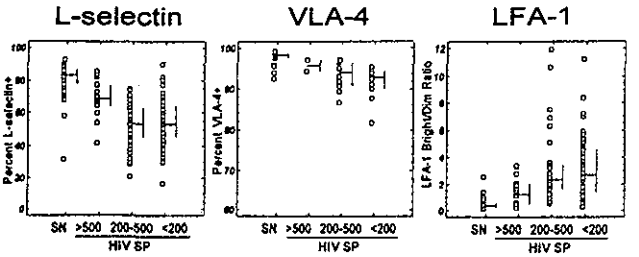


FIG. 2. Expression of L selectin, VLA-4, and LFA-1 on CD3$^+$ lymphocytes by HIV status and CD4$^+$-lymphocyte count category. SN, seronegative; HIV SP, HIV seropositive. The bars represent the medians (horizontal) and the 25th and 75th percentiles (vertical).

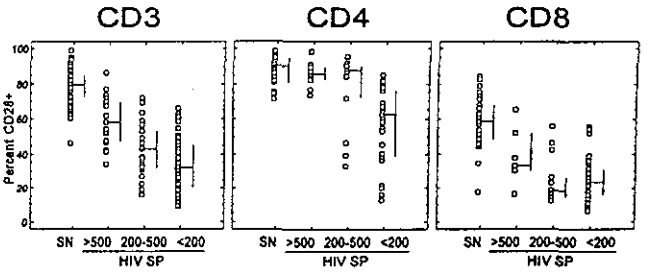


FIG. 3. Expression of CD28$^+$ on CD3$^+$-, CD4$^+$-, and CD8$^+$-lymphocyte subsets by HIV status and CD4$^+$-lymphocyte count category. SN, seronegative; HIV SP, HIV seropositive. The bars represent the medians (horizontal) and the 25th and 75th percentiles (vertical).

with lower CD4$^+$ lymphocyte counts. The proportions of CD4$^+$ lymphocytes expressing CD28$^+$ were similar among HIV-seropositive individuals with CD4$^+$ counts of >500 and 200 to 500 cells/μl but were lower among those with CD4$^+$ lymphocyte counts of <200 cells/μl. There was no clear trend for the proportion of CD8$^+$ lymphocytes expressing CD28$^+$ by CD4$^+$ lymphocyte count.

The percentages of CD3$^+$ CD28$^+$ lymphocytes and CD3$^+$ L selectin$^+$ lymphocytes were positively correlated (Spearman coefficient = 0.86; $P < 0.0001$), as shown in Fig. 4a. The percentage of CD3$^+$ CD28$^+$ lymphocytes and the CD3$^+$ LFA-1^{bright} lymphocyte/CD3$^+$ LFA-1^{dim} lymphocyte ratio were negatively correlated (Spearman coefficient = −0.92; $P <$ 0.00001), as shown in Fig. 4b.

DISCUSSION

The results of this study suggest that the expression of L selectin and VLA-4 is decreased and the expression of ICAM-1 and LFA-1 is increased on CD3$^+$ lymphocytes during HIV infection. A limitation of the current study is that the use of two-color analysis makes the findings regarding the expression of molecular and activation markers more inferential than if three-color analysis were used. Large unclassified cells were included in the total lymphocyte count. In HIV-infected children, these cells can make up a large proportion of lymphocytes and skew the phenotypic analysis. However, most of the HIV-infected adults did not have a large proportion of large unclassified cells.

The decreased expression of L selectin in both CD4$^+$ and CD8$^+$ lymphocytes has been described in HIV-infected adults (1, 9). In HIV-infected children, a slight decrease in CD4$^+$ CD45RA$^+$ lymphocytes expressing L selectin has been described (18, 19), and CD8$^+$ lymphocytes expressing both L selectin and CD45RA are markedly decreased (20). CD8$^+$ L selectin$^-$ lymphocyte counts are elevated in HIV-infected adults and predict progression to AIDS (10). The decreased expression of L selectin on CD3$^+$ lymphocytes suggests that leukocyte rolling along the vascular endothelium and lymphocyte circulation may be impaired during HIV infection.

In this study, HIV-infected individuals had significantly lower percentages and absolute counts of CD3$^+$ lymphocytes expressing VLA-4 than did HIV-seronegative individuals, and advanced HIV infection was associated with a small decrease in CD3$^+$ VLA-4$^+$ lymphocytes. VLA-4 mediates T-cell adhesion to the vascular endothelium via two ligands, vascular cell adhesion molecule-1 (VCAM-1) and fibronectin (CS-1 sequence), and VLA-4 also plays a role in T-cell costimulation (21). Relatively little is known about VLA-4 on T lymphocytes during HIV infection.

The percentages of CD3$^+$ lymphocytes expressing ICAM-1 were higher in HIV-infected individuals than in HIV-seronegative individuals in the present study, although the absolute numbers in the two groups were not significantly different. One limitation of the present study was the small sample size of those individuals in which ICAM-1 was measured. ICAM-1 is a member of the immunoglobulin gene superfamily and appears to play a major role in monocyte-lymphocyte communication (15) as well as in the binding of lymphocytes to the vascular endothelium. No differences were noted in the expression of ICAM-1 on monocytes between HIV-infected and HIV-seronegative individuals (15).

Our finding of increased expression of LFA-1 on CD3$^+$ lymphocytes in advanced HIV disease corroborates the findings of previous studies in which LFA-1 expression was upregulated in individuals with more advanced HIV disease (17, 24). Increased LFA-1 expression may facilitate extravasation

TABLE 2. Expression of CD28$^+$ on T lymphocyte subsets

Subset	Cells expressing CD28$^+$ [median (25th-75th percentile)]									
	HIV-seronegative adults					HIV-seropositive adults				
	n^a	No. (cells/μl)	*P* value	%	*P* value	n^a	No. (cells/μl)	*P* value	%	*P* value
CD3$^+$ CD28$^+$	49	1,228 (1,074-1,348)	0.0001	79.7 (72.4-85.6)	0.0001	100	377 (155-578)	0.0001	37.9 (29.5-52.7)	0.0001
CD3$^+$ CD28$^-$		305 (215-425)	0.0005				492 (245-853)	0.0005		
CD4$^+$ CD28$^+$	27	807 (723-1,015)	0.0001	90.6 (81.3-95.3)	0.0002	44	66 (17-364)	0.0001	78.6 (58.3-87.3)	0.0002
CD4$^+$ CD28$^-$		88 (46-183)	0.0004				26 (13-53)	0.0004		
CD8$^+$ CD28$^+$	27	281 (208-357)	0.002	58.5 (48.5-67.5)	0.0001	44	156 (91-300)	0.002	23.2 (16.1-33.9)	0.0001
CD8$^+$ CD28$^-$		198 (162-252)	0.0001				430 (248-803)	0.0001		

[a] *n*, number (*n*) of patients.

248

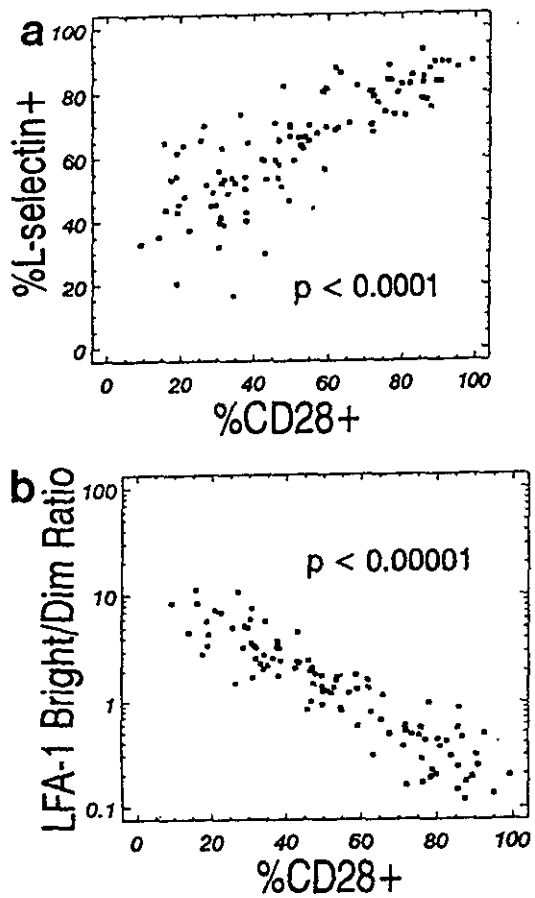


FIG. 4. (a) Percentage of $CD3^+$ $CD28^+$ lymphocytes expressing L selectin (Spearman correlation coefficient = 0.86; $P < 0.0001$). (b) Percentage of $CD3^+$ $CD28^+$ lymphocytes versus LFA-1^{bright}/LFA-1^{dim} ratio (Spearman correlation coefficient = 0.92; $P < 0.00001$).

of $CD3^+$ through the vascular endothelium into tissues during HIV infection. LFA-1 expression is also increased in monocytes during HIV infection (15), and in vitro studies show that there is a twofold increase in LFA-1 expression on HIV-infected monocytes (6). LFA-1 is incorporated into retroviruses from host cells. Increased expression of LFA-1 by $CD3^+$ lymphocytes could also contribute to more efficient virus infection by providing an additional ligand-receptor interaction between cell and virus. Recently it has been demonstrated that monoclonal antibodies to LFA-1 increase plasma neutralization of HIV (11).

The present study corroborates the finding that advanced HIV infection is associated with loss of both $CD4^+$ and $CD8^+$ lymphocytes expressing the CD28 receptor (3, 5). HIV infection of CD4 lymphocytes appears to down-regulate the expression of CD28 (12). The CD28 molecule on $CD4^+$ and $CD8^+$ lymphocytes interacts with the B7 molecule on antigen-presenting cells to generate signals for the production of interleukin 2 and cell proliferation, and interleukin 2 production appears to be limited to $CD8^+$ $CD28^+$ lymphocytes (2). $CD8^+$ $CD28^+$ lymphocytes appear to play a role in noncytotoxic activity that inhibits HIV replication (14), while $CD8^+$ $CD28^-$ lymphocytes have HIV-specific cytotoxic activity (8, 25). There was a high correlation between the percentages of $CD3^+$ L $selectin^+$ and $CD3^+$ $CD28^+$ lymphocytes in the present study, suggesting that $CD3^+$ $CD28^-$ lymphocytes probably do not express L selectin. The present study showed a negative correlation between the percentages of $CD3^+$ $CD28^+$ lymphocytes and $CD3^+$ LFA-1^{bright}/$CD3^+$ LFA-1^{dim} lymphocyte ratio, suggesting that $CD3^+$ $CD28^+$ lymphocytes do not express LFA-1.

ACKNOWLEDGMENTS

This research was supported by the National Institutes of Health (NS26643, AI76234, RR00722, HD30042).

We acknowledge the assistance of Marcia Hornsberger, Tish Nance-Sproson, Deneen Esposito, Brian Yost, and Julie Grim in the collection of samples and patient information and thank Joseph Margolick for critical comments.

REFERENCES

1. **Bass, H. Z., P. Nishanian, W. D. Hardy, R. T. Mitsuyasu, E. Esmail, W. Cumberland, and J. L. Fahey.** 1992. Immune changes in HIV-1 infection: significant correlations and differences in serum markers and lymphoid phenotypic antigens. Clin. Immunol. Immunopathol. 64:63–70.
2. **Brinchmann, J. E., J. H. Dobloug, B. H. Heger, L. L. Haaheim, M. Sannes, and T. Egeland.** 1994. Expression of costimulatory molecule CD28 on T cells in human immunodeficiency virus type 1 infection: functional and clinical correlations. J. Infect. Dis. 169:730–738.
3. **Caruso, A., A. Cantalamessa, S. Licenziati, L. Peroni, E. Prati, F. Martinelli, A. D. Canaris, S. Folghera, R. Gorla, A. Balsari, et al.** 1994. Expression of CD28 on $CD8^+$ and $CD4^+$ lymphocytes during HIV infection. Scand. J. Immunol. 40:485–490.
4. **Centers for Disease Control and Prevention.** 1992. Guidelines for the performance of $CD4^+$ T-cell determinations in persons with human immunodeficiency virus infection. Morbid. Mortal. Weekly Rep. 41(RR-8):1–17.
5. **Choremi-Papadopoulou, H., V. Viglis, P. Gargalianos, T. Kordossis, A. Iniotaki-Theodoraki, and J. Kosmidis.** 1994. Downregulation of CD28 surface antigen on $CD4^+$ and $CD8^+$ T lymphocytes during HIV-1 infection. J. Acquired Immune Defic. Syndr. 7:245–253.
6. **Dhawan, S., B. S. Weeks, C. Soderland, H. W. Schnaper, L. A. Toro, S. P. Asthana, I. K. Hewlett, W. G. Stetler-Stevenson, S. S. Yamada, K. M. Yamada, and M. S. Meltzer.** 1995. HIV-1 infection alters monocyte interactions with human microvascular endothelial cells. J. Immunol. 154:422–432.
7. **Feinberg, M. B.** 1996. Changing the natural history of HIV disease. Lancet 348:239–246.
8. **Fiorentino, S., M. Dalod, D. Olive, J. G. Guillet, and E. Gomard.** 1996. Predominant involvement of $CD8^+$ $CD28^-$ lymphocytes in human immunodeficiency virus-specific cytotoxic activity. J. Virol. 70:2022–2026.
9. **Giorgi, J. V., and R. Detels.** 1989. T-cell subset alterations in HIV-infected homosexual men: NIAID Multicenter AIDS Cohort Study. Clin. Immunol. Immunopathol. 52:10–18.
10. **Giorgi, J. V., Z. Liu, L. E. Hultin, W. G. Cumberland, K. Hennessey, and R. Detels.** 1993. Elevated levels of $CD38^+$ $CD8^+$ T cells in HIV infection add to the prognostic value of low $CD4^+$ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. J. Acquired Immune Defic. Syndr. 6:904–912.
11. **Gomez, M. B., and J. E. Hildreth.** 1995. Antibody to adhesion molecule LFA-1 enhances plasma neutralization of human immunodeficiency virus type 1. J. Virol. 69:4628–4632.
12. **Haffar, O. K., M. D. Smithgall, J. G. Wong, J. Bradshaw, and P. S. Linsley.** 1995. Human immunodeficiency virus type 1 infection of CD4' T cells down-regulates the expression of CD28: effect on T cell activation and cytokine production. Clin. Immunol. Immunopathol. 77:262–270.
13. **Hynes, R. O.** 1992. Integrins: versatility, modulation, and signalling in cell adhesion. Cell 69:11–25.
14. **Landay, A. L., C. E. Mackewiez, and J. A. Lévy.** 1993. An activated $CD8^+$ T cell phenotype correlates with anti-HIV activity and asymptomatic clinical status. Clin. Immunol. Immunopathol. 69:106–116.
15. **Locher, C., G. Vanham, L. Kestens, M. Kruger, J. L. Ceuppens, J. Vingerhoets, and P. Gigase.** 1994. Expression patterns of Fcγ receptors, HLA-DR and selected adhesion molecules on monocytes from normal and HIV-infected individuals. Clin. Exp. Immunol. 98:115–122.
16. **Margolick, J. B., A. Muñoz, A. D. Donnenberg, L. P. Park, N. Galai, J. V. Giorgi, M. R. O'Gorman, J. Ferbas.** 1995. Failure of T-cell homeostasis preceding AIDS in HIV-1 infection. Multicenter AIDS Cohort Study. Nat. Med. 1:674–680.
17. **Ng, T. T., C. Guntermann, K. E. Nye, J. M. Parkin, J. Anderson, J. E. Norman, and W. J. Morrow.** 1995. Adhesion co-receptor expression and intracellular signalling in HIV disease: implications for immunotherapy. AIDS 9:337–343.
18. **Plaeger-Marshall, S., P. Hultin, J. Bertolli, S. O'Rourke, R. Kobayashi, A. L. Kobayashi, J. V. Giorgi, Y. Bryson, and E. R. Stiehm.** 1993. Activation and

249

differentiation antigens on T cells of healthy, at-risk, and HIV-infected children. J. Acquired Immune Defic. Syndr. 6:984–993.

19. **Plaeger-Marshall, S., V. Isacescu, S. O'Rourke, J. Bertolli, Y. J. Bryson, and E. R. Stiehm.** 1994. T cell activation in pediatric AIDS pathogenesis: three-color immunophenotyping. Clin. Immunol. Immunopathol. 71:19–26.
20. **Rabin, R. L., M. Roederer, Y. Maldonado, A. Petru, L. A. Herzenberg, and L. A. Herzenberg.** 1995. Altered representation of naive and memory CD8 T cell subsets in HIV-infected children. J. Clin. Invest. 95:2054–2060.
21. **Sato, T., K. Tachibana, Y. Nojima, N. D'Avirro, and C. Morimoto.** 1995. Role of the VLA-4 molecule in T cell costimulation. Identification of the tyrosine phosphorylation pattern induced by the ligation of VLA-4. J. Immunol. 155:2938–2947.
22. **Springer, T. A.** 1990. Adhesion receptors of the immune system. Nature 346:425–434.
23. **Tedder, T. F., D. A. Steeber, A. Chen, and P. Engel.** 1995. The selectins: vascular adhesion molecules. FASEB J. 9:866–873.
24. **Vermot Desroches, C., and D. Rigal.** 1990. Leukocyte function-associated antigen-1 expression on peripheral blood mononuclear cell subsets in HIV-1 seropositive patients. Clin. Immunol. Immunopathol. 56:159–168.
25. **Vingerhoets, J. H., G. L. Vanham, L. L. Kestens, G. G. Penne, R. L. Colebunders, M. J. Vandenbruaene, J. Goeman, P. L. Gigase, M. De Boer, and J. L. Ceuppens.** 1995. Increased cytolytic T lymphocyte activity and decreased B7 responsiveness are associated with CD28 down-regulation on $CD8^+$ T cells from HIV-infected subjects. Clin. Exp. Immunol. 100:425–433.

250