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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 during calendar year 1997. These studies were supported, in whole or in part, by the "Vitamin A for Health" or "Micronutrient for Health" cooperative agreements with USAID.

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1997 PUBLICATIONS

VITAMIN A

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Infant feeding practices reflect antecedent risk of xerophthalmia in Nepali children

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Objective: To determine the relationship between infant feeding history and risk of xerophthalmia due to vitamin A deficiency (VAD) in early childhood.
Design: A case-control study of previously xerophthalmic and non-xerophthalmic children.
Setting: Rural lowland region of Nepal.
Subjects: One hundred and fifty-six children (aged 1–6 y old), half of whom previously had xerophthalmia due to vitamin A-deficiency, the other half matched by locale, age and the presence and age of a younger sibling ($n = 102$).
Methods: Xerophthalmia was determined by trained ophthalmic assistants on the basis of current Bitot's spots, corneal xerosis or report of night blindness. Infant feeding history was collected through a diet history method obtained from the mother of the focus child. Exploratory factor analysis was conducted to determine the presence of underlying patterns in infant feeding practices. Conditional logistic regression was used to estimate odds ratios.
Results: Mothers of control children tended to have a higher level of education ($P < 0.10$) and to have fewer children who had died ($P < 0.10$) than mothers of case children. Feeding of meat (OR = 0.09, CI = 0.01–0.70) or fish (OR = 0.41, CI = 0.17–0.99) with liver, eggs (OR = 0.11, CI = 0.01–0.88) and mango (OR = 0.28, CI = 0.13–0.60) were protective in association with xerophthalmia in early childhood. Factor analysis uncovered several distinct patterns in infant feeding, which varied by age of the infant. Only the 'animal flesh' feeding pattern (factor), practiced in the second year of life, proved significantly protective from xerophthalmia (OR = 0.43, CI = 0.20–0.94). Feeding patterns of younger children closely paralleled those of their older siblings with and without VAD.
Conclusions: The study supports the hypothesis that infant dietary practices can influence subsequent risk for VAD. Our findings emphasize the importance of introducing vitamin A-rich foods during weaning to reduce the risk of VAD-associated xerophthalmia in the later preschool years.
Sponsorship: The project is 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, US Agency for International Development, with additional support from Task Force Sight and Life (Roche, Basel), and NIH shared instrument grant No. S10-RR 04060.
Descriptors: infant feeding; diet history; xerophthalmia; vitamin A deficiency; Nepal

Introduction

There is a large literature linking a low, current intake of vitamin A to xerophthalmia in preschool-aged children (Blankhart, 1967; Pepping *et al*, 1989 Solon *et al*, 1978; Sommer & West, 1996; Tarwotjo *et al*, 1982). The evidence to date is believed to reflect a pattern of chronically low vitamin A intakes. Yet, beyond a propensity to wean early (Khatry *et al*, 1995; Tarwotjo *et al*, 1982) little is known about complementary feeding patterns during early infancy of high-risk children prior to developing clinically evident vitamin A deficiency (VAD). Secondly, given that younger siblings are at a greatly increased risk of developing xerophthalmia (Katz *et al*, 1993) and that risk factors tend to cluster within families (Sommer & West, 1996), it is of interest to understand early dietary patterns of pre-xerophthalmic siblings in order to identify inadequacies of intake and develop more comprehensive dietary counseling strategies for high-risk families and their children.

The most accurate means of addressing these questions would include costly longitudinal studies spanning early infancy through school-age. Therefore, much of the literature linking infant feeding patterns and childhood nutritional status has been indirect (Dettwyler & Fishman, 1992), drawing inferences through the examination of different aged children within the same household. Murphy *et al* (1995) observes that nutrient adequacy tends to be a household characteristic for toddlers and school children. From a study of 94 toddler-schoolchild pairs, siblings at high risk of nutrient inadequacy could be identified with high levels of sensitivity and specificity from an indicator child. A similar situation exists with vitamin A deficiency, as siblings of cases are 7–13 times more likely to have, or develop, xerophthalmia once they enter the high-risk age group than children living in households without a xerophthalmic sibling (Katz *et al*, 1993). In Nepal, dietary patterns of xerophthalmic children and their younger siblings were found to be comparable, both having

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low intakes of vitamin A rich foods (Shankar *et al*, 1996), suggesting that the chronic dietary risk of VAD begins early in life and concentrates in high risk households. As Sommer & West (1996) observe, 'For many children, a weaning diet deficient in vitamin A predicts a vitamin A deficient diet through the rest of early childhood, and possibly beyond.'

Although most studies examining dietary patterns of children with night blindness have found significant differences in consumption of preformed vitamin A (Shankar *et al*, 1996; Tarwotjo *et al*, 1982) and provitamin A food sources (Pepping *et al*, 1989; Solon *et al*, 1978; Tarwotjo *et al*, 1982), little evidence exists of the linkage between infant dietary intake of vitamin A food sources and later childhood xerophthalmia. An exception is a study by Mele *et al* (1991), which looked at xerophthalmic Indonesian children from 0–5 y (96% > 2 years) and matched controls, which found significantly reduced risk of xerophthalmia in children whose mothers reported feeding them vitamin A-rich foods regularly during the first year of weaning.

Timing of introduction of key foods may also be determinant of later childhood feeding and risk. Xerophthalmic children in Malawi were reported to have begun the weaning process, on average, one month earlier than non-xerophthalmic controls (3 *vs* 4 months of age) (West *et al*, 1986). The infant feeding process can be divided into different stages, each associated with patterns of feeding and interaction between care giver and child. In Nepal, Gittelsohn (1991) found that for children less than a year, 72% of the infant-care giver feeding interactions involved breast-feeding. Zeitlin & Ahmed (1995) looked at infant feeding in rural Bangladesh over three infant age periods 5–12, 13–18 and 19–23 months and found that 'growth stasis' occurs primarily during the second period, associated with decreased breast-feeding time and less food intake.

Studies of infant feeding practices conducted within Nepal reveal a great deal of diversity in infant feeding practices by ethnic and caste group (Gittelsohn, 1989; Graves, 1984; Krantz, 1979; Levine, 1987; Panter-Brick, 1991; Reissland & Burghart, 1988). In general, common patterns identified include lack of feeding of colostrum to the infant, generally long duration of breast-feeding (2–3 y), lack of preparation of specialized weaning foods (such as cereal paps), and relatively late introduction of solid foods (after six months of age).

This study describes feeding patterns during the first two years of life using infant feeding histories of 156 children aged 1–6y, half of whom were diagnosed with xerophthalmia, the other half selected as age-village-younger sibling matched non-xerophthalmic controls in rural Nepal. Dietary histories are also examined for the next younger sibling (if any) in case and control households to determine whether inter-sibling feeding patterns are correlated. Using dietary histories in high-risk (for xerophthalmia) and low-risk households (non-xerophthalmic controls) we explore the relationship between infant diet and the presence of clinical vitamin A deficiency in early childhood.

Methods

Study design

A case-control study was conducted in three village development committees (VDCs) totaling approximately 15 000 population in Sarlahi District, located in the central lowland region of Nepal. Children with clinical signs of xerophthalmia or 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 1–6 y. All case children 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 in any sibling or the child's mother. Controls were matched to case households based on geographic proximity, ages of case and potential controls (±3 months), and ages of the next younger siblings (±4 months), if present in the case household. Five cases either declined to participate or migrated from the region. The age distribution of the 81 case children were as follows: ages 1–2 y (6 cases), ages 3–4 y (38 cases), ages 5–6 y (37 cases). An additional three case households were omitted due to migration of their control counterpart, resulting in a total of 78 case-control pairs included in the analysis.

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, MD.

Ethnographic data were collected for a period of four months on the following topics: identifying local foods given to infants and young children aged less than two years; determining key stages in the life of the infant as identified by the mother; and identifying appropriate frequency of feeding units. These data were used to develop an infant diet history instrument which asked the caregiver to describe feeding of 21 key foods for five different time periods: the first three days of life, the remainder of the first month of life, months 2 through 6, months 7 through 12 and the second year of life. During each time period, the caregiver was asked to report the frequency of feeding to the infant as (a) never, (b) infrequently (3 times per month or only to taste), (c) somewhat regularly (>3 times per month to 2 times per week), or (d) regularly (every day or every other day). Portion sizes were not obtained. Additional questions identified primary decision-makers and sources of advice for the caregiver in feeding the infant colostrum and other non-breastmilk foods.

The infant diet history was obtained once for the study child and once for the next youngest sibling, if any, in all case and control households. This instrument was administered 12–18 months after the initial diagnosis of xerophthalmia from the February 1992 ocular survey. The infant diet history was performed by two trained Nepali female data collectors. In all households, the primary respondent was the mother of the child. During the interview, mothers were asked to sit together with the child whose dietary history was being ascertained to help improve recall and avoid confusion with other children.

Data analysis

Frequency of consumption of the 21 key foods examined in the infant diet history was calculated over the two-year period of the infant diet history. Exploratory factor analysis was used to identify underlying patterns of infant feeding responsible for covariation in the data. This method has been applied to examine diets based on food frequency results in order to identify dietary patterns (Barker *et al*, 1990; Randell *et al*, 1990; Schwerin *et al*, 1982).

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Factor analysis calculates squared multiple correlations as prior communality estimates (Hatcher, 1994). The principal factor method was used to extract the factors, followed by an oblique (promax) rotation (An oblique rotation is preferred when factors to be extracted are considered to be interrelated). Separate factor analyses were run for ages one month, 2–6 months, 7–12 months, 13–24 months and 1–24 months. A combination of scree test (a plot of the eigenvalues of the factors) and assessment of proportion of variance accounted by the factors were utilized to determine the number of factors to be retained for rotation. In interpreting the rotated factor pattern, a selected food was considered to load on a given factor if the factor loading was 0.30 or greater for that factor, and was less than 0.30 for all other factors. No food item was permitted to load at the 0.30 level in more than one factor. Factor scores for each dietary pattern identified were computed for each infant by multiplying the factor weight of each food included in the pattern by the standardized values of the reported frequency of use, and summing over all foods in the factor. Factors were named based on our understanding of infant feeding in the setting and from the preliminary ethnographic data.

Many of the factors created include only two foods, representing a modification of the standard approach to factor analysis. We feel an exception to the standard approach is justified as mothers were presented with a relatively small number of distinct foods.

Matched-pair odds ratios (OR) and 95% confidence intervals (CI) were calculated by conditional logistic regression to estimate the risk of not feeding or feeding less of a particular food item or according to a particular factor pattern of a case relative to a control. To improve the meaningfulness of the parameter estimates, all covariates were reduced to binary form. Food frequencies were dichotomized either at the median of the frequency distribution or according to the presence or absence of the food during the respective time interval. Factor covariates were dichotomized at the median of the sample distribution. Odds ratios were adjusted for socio-economic status (SES) which was a scaled variable assessing the number of different types of key material possessions owned. The final scale included eight items (in currently usable condition): (a) flashlights (1+), (b) watch (1+), (c) cycle (1+), (d) armoire (1+), (e) beds (2+), (f) rooms in the house (2+), (g) a roof made with tile or cement, and (h) irrigation pumpset. The Cronbach's alpha (Cronbach, 1951) for this scale was 0.78, indicating acceptable scale reliability, with a mean score of 3. Those households having a scale score equal or greater to the mean (3.0) were classified as having a higher SES (a value of 1 in regression equation), those below the mean were classified as of lower status (value of 0).

Results

Background information

Table 1 presents a comparison of basic demographic indicators in study households. There were no significant differences between the mothers of case and control children in terms of age, number of children born alive, paternal education and household size. There appears to be a trend for more child death in case than control households ($P < 0.10$). Control child mothers tended to have greater amounts of education than case child mothers ($P < 0.10$), although the average number of years was much less than one year for both groups. There were four households where the father of the focus child was not living, all of which are case households. More control households (27.0%) consisted of two or more families living together than case households (9.8%). There were no significant differences between case and control households in terms of religion or caste.

Sources of advice and decision-making regarding infant feeding

Mothers reported themselves and their mothers-in-law as the decision-makers regarding infant feeding, but the responses varied by case status. Mothers of cases reported themselves as the primary decision-maker (39% *vs* 26%, $P < 0.10$), while more control mothers relied on their mothers-in-law (35% *vs* 26%, $P > 0.10$). This distribution

Table 1 Basic demographics of case and control households ($n = 78$ case-control pairs)

Characteristics	*Case*		*Control*		*P-value*
	$\bar{x}$	*SE*	$\bar{x}$	*SE*	
Maternal age (y)	28.9	0.58	28.8	0.62	0.960
Paternal age (y)	33.7	0.75	33.8	0.78	0.920
Number of children born alive[a]	4.68	0.23	4.62	0.23	0.615
Number of children who died[b]	1.01	0.13	0.86	0.16	0.078
Maternal education (y)[c]	0.05	0.05	0.33	0.14	0.053
Paternal education (y)[d]	1.71	0.31	3.04	0.44	0.188
Household size[e]	6.80	0.28	7.55	0.31	0.336
	%	*n*	%	*n*	*P-value*
Religion					
Hindu	97.4%	76	93.6%	73	0.440
Muslim/Buddhist	2.6%	2	6.4%	5	
Hindu caste[f]					
high (Brahmin)	4.0%	3	5.6%	4	
middle (Chhetri/Viasya)	78.4%	58	80.6%	58	0.810
low (Sudra)	17.6%	13	13.9%	10	

[a] X^2: 3 or less *vs* 4+ live children.
[b] X^2: 0 *vs* 1+ dead children.
[c] X^2: 0 *vs* 1+ years of mother's education.
[d] X^2: 1 or less *vs* 2+ years of father's education.
[e] X^2: 6 or less *vs* 7+ household members.
[f] Of the 149 Hindu households, caste information was unavailable for 1 case and 2 control households.

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is consistent with the greater likelihood of control households being part of joint or extended households. Almost a quarter of the mothers reported that their husbands made the infant feeding decisions.

When asked to identify their main advisors in infant feeding, the most common responses were a neighbor (45%) or mother-in-law (32%). Mothers of cases were significantly more likely to report that they learned about infant feeding from their mother (15% *vs* 5%, $P < 0.05$) or were self-taught (8% *vs* 1%, $P < 0.05$), although these numbers are small.

Patterns of early feeding

Breast-feeding was evident for almost all focus children and their younger siblings throughout the first two years of life. Half of the study mothers reported giving their child colostrum, with no significant differences between cases and controls. More than 40% of study mothers fed their children colostrum because they claimed that they "did not know not to feed it to the child," while less than 5% of mothers gave colostrum for its health benefits. Of the 50% of mothers who reported not feeding their infant colostrum, half said others had told them not to feed it to their infant (25%). Some believed that colostrum was dirty (13%) and others thought that it caused the baby to have diarrhea (6%). Mothers of cases were significantly more likely than those of controls to report that they had avoided giving their child colostrum due to the fear it would cause vomiting (10% *vs* 1%). Although many mothers reported not feeding their infant colostrum, they also reported some breast-feeding of the child during the first three days of life which may indicate that many mothers feed their infants colostrum when they think they do not.

A majority of mothers (>85%) reported introduction of foods after six months of age. Early supplemental foods include a variety of adult meal foods, such as rice, vegetables, or fruits, however, the frequency of consumption was low (3 times per month). Regular consumption of these foods were reported in the second year of life.

Frequency of feeding of individual foods

Table 2 compares differences in feeding 21 different foods between case and control children in their first two years of life. Preformed vitamin A sources consumed during infancy were strongly associated with reduction of risk for xerophthalmia in later preschool years. Consumption of any meat (usually with some liver) in the first two years of life was associated with a 91% reduction in risk (OR = 0.09, CI = 0.01–0.70) of xerophthalmia. Consumption of fish (usually in small amounts) was associated with a 59% reduction in risk (OR = 0.41, CI = 0.17–0.99) for xerophthalmia. Of the various carotenoid sources considered, only mango intake was associated with significant protection against subsequent xerophthalmia (OR = 0.28, CI = 0.13–0.61), whereas dark green leafy vegetables (DGLV) intake was not (OR = 1.32, CI = 0.65–2.66). Consumption of animal milk was also greater in control households (OR = 0.56) although the confidence intervals included unity. Foods low in vitamin A content were not associated with significant differences in risk between case and control children.

Analyses comparing the infant feeding pattern of focus children with that of their younger siblings (age 24+ months at time of interview) shows remarkable conformity in the pattern of feeding (Table 3). As would be expected, the infant histories of younger siblings is highly correlated with that of their older counterparts in the same household. This finding is supported by correlations in sibling's frequency of consumption of specific, vitamin-A rich food items. Preformed vitamin A foods exhibited high correla-

Table 2 Case-control differences in frequency[a] of feeding of 21 key foods over first 24 months[b] ($n = 78$ age-matched case-control pairs)

Food consumption	*Cases* (%)	*Controls* (%)	*Adjusted[c] Odds Ratios*	*(95% CI)*
Vitamin A sources				
Mother's milk (0–24) daily	100	97	—	—
Meat (2–24) ever	85	97	0.09	(0.01–0.70)[d]
Fish (7–24) ever	69	82	0.41	(0.17–0.99)[d]
Mango (7–24) infrequently	37	64	0.28	(0.13–0.60)[d]
Animal milk (0–24) infrequently	40	51	0.55	(0.26–1.15)
DGLVs (7–24) infrequently	40	35	1.29	(0.64–2.59)
Papaya (7–24) infrequently	29	29	1.00	(0.49–2.05)
Other breast milk (0–24) ever	26	23	1.22	(0.51–2.95)
Eggs (7–24) infrequently	3	13	0.11	(0.01–0.88)[d]
Malt drink (7–24) ever	3	4	0.67	(0.11–3.99)
Powdered milk (0–24) ever	3	4	0.50	(0.05–5.51)
Non-vitamin A sources				
Sweet potato-white (7–24) ever	91	95	0.57	(0.17–1.95)
Rice and dal (7–24) regularly	69	72	0.88	(0.43–1.79)
Other vegetables (7–24) regularly	64	60	1.25	(0.59–2.67)
Traditional tonic (2–24) ever	51	46	1.24	(0.65–2.34)
Watery rice dal mix (7–24) infrequently	56	60	0.83	(0.42–1.65)
Unleavened wheat bread (7–24) regularly	45	46	0.93	(0.44–1.98)
Honey (0–24) infrequently	42	37	1.25	(0.65–2.41)
Banana (7–24) infrequently	38	36	1.13	(0.57–2.21)
Glucose water (7–24) ever	14	10	1.43	(0.54–3.75)
Broth (7–24) ever	12	13	0.86	(0.29–2.55)

[a]Infrequent consumption over the specified time period refers to >once a month to 2 times/week; regular consumption refers to 3 or more times per week.
[b]The period for which any specific food was eaten is designated as months in the () since many foods were not introduced until the seventh month.
[c]Odds ratios are adjusted for SES.
[d]$P < 0.05$.

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Table 3 Correlations in siblings' feeding patterns and reported frequency of consumption of selected food items ($n = 67$ focus child and younger sibling pairs[a])

Specific food items	*Correlation between siblings*	
	Spearman's rank correlation	*P value*
Preformed vitamin A sources		
Meat	0.38	0.0014
Fish	0.39	0.0012
Traditional tonic	0.38	0.0013
Animal milk	0.66	0.0001
Other breast milk	0.50	0.0001
Eggs	0.53	0.0001
Carotenoid sources		
Mango	0.54	0.0001
DGLVs	0.33	0.0064
Papaya	0.14	0.2687
Non-vitamin A foods		
Sweet potato (white)	0.44	0.0002
Rice and dal	0.42	0.0004
Other vegetables	0.25	0.0454
Watery rice and dal mix	0.45	0.0001
Unleavened wheat bread	0.20	0.1020
Honey	0.24	0.0464
Banana	0.22	0.0784

[a]Only younger siblings age >24 months at time of interview used.

tions in reported two-year consumption frequencies between siblings. Carotenoid sources were somewhat less consistently correlated, perhaps reflecting the influence of seasonal variability in their availability.

Results of factor analysis

The infant feeding patterns identified in this study using factor analysis are presented in Table 4. Factors were extracted separately for month 1, months 2–5, months 7–12, months 13–24 and all 24 months. Case-control comparisons of these feeding patterns are summarized in Table 5.

Month 1 feeding patterns

Only one factor was extracted for this time period. Most of the 21 foods were not included in the factor as they were never given to such young infants. Breastmilk does not appear in the factor because all children received it several times daily. The factor, labeled 'other milks,' consists of other women's breastmilk, animal milk and honey. No differences were observed between case and control children in the frequency of feeding for this scale.

Table 5 Adjusted[a] matched-pair odds ratios for xerophthalmia based on infant feeding patterns (factors) ($n = 78$ matched case-control pairs)

Age period	*Factors*	*Adjusted odds ratio*	*95% CI*
0–1 month	Other milks	1.17	(0.75–1.83)
2–6 months	Side dishes	1.23	(0.72–2.09)
	Snacks	1.46	(0.71–3.02)
7–12 months	Regular meal items	1.09	(0.68–1.74)
	Snacks	1.15	(0.76–1.75)
	Powdered supplement	0.87	(0.46–1.65)
	Sick foods	0.78	(0.42–1.45)
13–24 months	Infant foods	0.58	(0.16–2.07)
	Animal flesh	0.43	(0.20–0.94)[b]
	Regular meal items	0.75	(0.43–1.27)
0–24 months	Regular meal items	0.94	(0.59–1.51)
	Breastmilk	2.77	(0.65–11.71)
	Animal flesh	0.57	(0.35–0.92)[b]
	Sick foods	0.95	(0.55–1.64)

[a]Odds ratios adjusted for SES (as determined by material possessions score).
[b]$P < 0.05$.

Months 2–6 feeding patterns

Two factors were extracted for months 2–6. The first factor, labeled 'side dishes' includes vegetable dishes and meat dishes. In the 2–6 month age period, side dishes are occasionally given to small children to taste. Of those children who reportedly received these foods at this age, all were fed rarely or just to taste. The second factor, labeled 'snacks' includes sweet potatoes and honey. About a third of the infants reportedly received honey at least occasionally during this time period and a few received it once a week or so. No differences were observed between

Table 4 Exploratory factor analysis results for infant feeding patterns ($n = 156$ case and control focus children)

Age of infant	*Factor 1*	*Factor 2*	*Factor 3*	*Factor 4*
1 month	Other milks: Other breastmilk, Animal milk, Honey			
2–6 months	Side dishes: Vegetable dishes, Meat dishes	Snacks: Sweet potatoes, Honey		
7–12 months	Regular meal items: Wheat bread, Vegetable dishes, DGLVs, Rice and dal, Sweet potatoes, Meat dishes	Snacks: Broth, Mango	Powdered supplements: Glucose water, Powdered milk	Sick foods: Malt drink, Eggs
13–24 months	Infant foods: Breastmilk Vegetable dishes, Not powdered milk	Animal flesh: Meat, Fish	Regular meal items: Papaya, Wheat bread, Animal milk, DGLVs, Water rice dal mix	
1–24 months	Regular meal items: DGLVs, Vegetable dishes, Papaya, Watery rice dal mix, Broth, Wheat bread	Breastmilk: Breastmilk, Not powdered milk	Animal flesh: Meat, Fish	Sick foods: Malt drink, Traditional tonics

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case and control children in the frequency of feeding for either of these scales.

Months 7–12 feeding patterns
Four factors were extracted for months 7–12. The first factor, labeled 'regular meal items' includes unleavened wheat bread, vegetable dishes, dark green leafy vegetables, rice and dal (mixed together), sweet potatoes and meat dishes. Most of these foods were given rarely or to taste to infants in this age group. The exception was rice and dal: over 70% of infants received the staple meal regularly.

The three other factors extracted include: 'snacks' (broth and mango), usually given only rarely or to taste; 'powdered supplements' (glucose water and powdered milk), and 'sick foods' (malt drink and eggs). No significant differences were observed between cases and control for any of these patterns.

Months 13–24 feeding patterns
Three factors were extracted for months 13–24. The first factor, labeled 'infant foods', includes breastmilk, vegetable dishes and no powdered milk. In the second year, most infants (98.7%) are still being breast-fed regularly. The second factor, 'animal flesh' includes meat and fish (including liver). Over 90% of study children reportedly received these foods during the second year of life—the majority of them infrequently. The third factor, labeled 'regular meal items' includes papaya, roti, animal milk, DGLVs and watery rice-dal mix. Significant differences between cases and controls were observed for the 'animal flesh' intake which was associated with a 57% reduction in risk for xerophthalmia for this age group (OR = 0.43, CI = 0.20–0.94).

Overall feeding patterns in first two years
When all infant feeding periods are combined (weighted for duration), four factors were extracted, including (1) 'regular meal items' (DGLVs, vegetable dishes, papaya, watery rice dal mix, broth, unleavened wheat bread), (2) 'breastmilk' (breastmilk, no powdered milk), (3) 'animal flesh' (meat and fish), and (4) 'sick foods' (malt drink and traditional tonics). As in the 13–24 month age group, the only significant infant feeding pattern differentiating between cases and controls is the 'animal flesh' pattern. More frequent feeding of these foods was associated with an odds ratio of 0.57 (CI = 0.36–0.92).

Discussion and conclusions

Households where there is at least one xerophthalmic child have been shown to live in relatively greater poverty, with less land and fewer material possessions (Cohen, 1985; Khatry *et al*, 1995). Within these households, more than one household member may be at risk, including the mother (Katz *et al*, 1995) and other siblings (Katz *et al*, 1993). Dietary behaviors (those low in vitamin A-rich food sources) during infancy may be a contributing factor to clinical deficiency by early childhood. From these data it appears that feeding patterns during infancy, as early as seven months, are associated with children's risk of developing xerophthalmia later in the preschool years. This relationship is primarily related to the feeding of preformed vitamin A sources, namely meat and fish, both usually with liver. While these foods are given to infants in very small quantities, consumption of these foods may be indicative of a diet that is of better nutrient quality, enough to swing the balance away from clinical manifestations of VAD later in life. These results are evident despite the problems inherent to conducting a recall of feeding behaviors of two to five years in the past.

Distinct patterns in the feeding of infants can be identified in this population. These patterns may be generally divided into five main clusters of foods: milks, regular meal items, sick foods, animal flesh and snacks. The foods that make up these food groups vary by age of the infant. Very few of these patterns appear to differentiate between xerophthalmic children and their non-xerophthalmic peers. Further work needs to be done to ascertain the value of these categories of infant diet in terms of their effect on other health outcomes.

On the other hand, describing these food groupings appear to be helpful in identifying the different stages of the infant feeding process in this setting. In the first six months of life, most children are primarily breast-fed, and there are very few other food clusters of importance. The two major groups that exist are limited to infrequent tasting of certain food items and side dishes. Feeding of regular meal items does not appear to be important until the 7–12 month age period. Here, the early 'side dishes' factor broadens to include staples, DGLVs and potatoes, and the composition of snacks evolves as well. Two other important food groups appear to come into play: powdered supplements and foods given especially to sick children. Both of these food groups are noted by parents as remedies to children who experience greater morbidity due to the introduction of weaning foods.

There is relatively little variation in feeding during the first year of life as nearly all children are breast-fed; mother's tend to report not feeding colostrum; 85% are reputed to have been exclusively breast-fed over months 2–6 and 11.5% have only one additional food item. These findings are in accordance with other ethnographic studies conducted with women in this part of Nepal. Reissland & Burghart (1988) found a universal discarding of colostrum by Maithili-speaking mothers living in Janakpur. While half of our mothers reportedly avoided giving their infant colostrum, the other half did not, indicating a difference between belief and stated behavior in this population.

Significant variation in feeding patterns begins during the second year of life, when the child begins consuming small portions of the household meal. Based on a one-time remote recall of early infant diet, our findings indicate that consumption of preformed vitamin A foods, particularly meat (with liver), is important in differentiating later risk of xerophthalmia. Although direct comparisons between past infant diet and present childhood diet (which were obtained using four 7 d food frequencies of the preschool aged child) (Shankar *et al*, 1996) are not possible due to differences in the data collection methods used, it appears that this dietary pattern continues to distinguish case from control children throughout early childhood.

Many studies have shown that current or recent diet of children with xerophthalmia is low in vitamin A-rich foods when compared to non-xerophthalmic, similar aged children. Our study indicates that, in this setting, dietary risk factors for xerophthalmia in young children extend back into the infant feeding period.

These findings suggest that efforts to improve the dietary quality of young children should extend to infants in areas of high risk of VAD. In rural Nepal, early infant dietary intake of vitamin A-rich foods cannot be said to be directly responsible for later childhood xerophthalmia, but do

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appear to indicate greater risk for childhood diets that are deficient in vitamin A. The evidence from our study and others strongly suggest that in households with at least one xerophthalmic preschool aged child, nutrition intervention programs should be inclusive of infants and other young (prexerophthalmic) children. Appropriate dietary strategies should target all infants and children in high-risk households.

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VITAMIN A DEFICIENCY AMONG FORMER AND CURRENT INJECTING DRUG USERS IN BRONX, NEW YORK

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ABSTRACT

The prevalence of vitamin A deficiency was studied cross-sectionally in 135 HIV-infected and 45 HIV-uninfected high risk former and current injecting drug users (IDUs) in the Bronx, New York. Vitamin A levels were similar by serostatus: 35% (47/135) of HIV-seropositive and 33% (15/45) of HIV-seronegative subjects had serum vitamin A levels consistent with deficiency (<1.05 μmol/L); 17% HIV-seropositive and 11% HIV-seronegative subjects had severe vitamin A deficiency (<0.70 μmol/L). Among the HIV seropositive subjects women were significantly more likely to be vitamin A deficient (29%, 27/92 of males versus 40%, 17/43 of females, $p=0.05$). Median serum vitamin A was associated with $CD4^+$ levels previously found to be prognostic of disease progression in this cohort; 0.81 μmol/L versus 1.19 μmol/L for <150 and ≥ 150 cells/mm^3 respectively, $p= 0.02$. Serum vitamin A levels were significantly associated with hemoglobin levels in HIV- infected individuals only (Mantel Haenszel trend, $p=0.05$). Further study of vitamin A deficiency and infectious disease morbidity in HIV-infected and uninfected high risk IDUs may be warranted given the high prevalence of vitamin A deficiency among this cohort of IDUs and the association of vitamin A with infectious disease morbidity.
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Key Words: Vitamin A deficiency, Injecting Drug Use, HIV infection

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INTRODUCTION

Vitamin A is an essential micronutrient for immunity, growth, reproduction, maintenance of epithelial surfaces and vision (1). Among the micronutrients, vitamin A plays a central role in normal immune function (2), and vitamin A deficiency has long been associated with infections and increased morbidity and mortality (3-7). Vitamin A deficiency is characterized by a wide variety of immune alterations, including loss of $CD4^+$ T-cells from lymph nodes and peripheral circulation, and impaired antibody responses (2,8,9). Characteristics of HIV infection are loss of circulating $CD4^+$ T-cells and impaired antibody responses (8-9). These immune abnormalities have also been reported in IDUs without HIV infection(10). Vitamin A deficiency may be of particular importance in HIV infection because the occurrence of the two conditions may be additive or synergistic in causing immunodeficiency with the potential for more severe clinical outcome.

Vitamin A deficiency has been reported in various populations in the United States infected with HIV, including homosexual men and injection drug users (IDUs)(11-15). The reported prevalence of vitamin A deficiency varies, ranging from 11%-29% with the higher rates in studies of patients with AIDS or AIDS related complex. Vitamin A deficiency has been reported to occur in approximately 15% of HIV-infected injection drug users in Baltimore, Maryland (16). We conducted a cross-sectional study to determine the extent of vitamin A deficiency among HIV-infected and HIV- uninfected IDUs in the Bronx, New York City.

METHODS

The study sample was selected from IDUs who are participating in an ongoing longitudinal study of the natural history of HIV infection in current and former injecting drug users attending a methadone maintenance program in the Bronx, New York. In January 1994, a random sample of 135 HIV seropositive and 45 HIV seronegative subjects with research visits in the previous 18 months was selected for this cross-sectional study. Pregnant women were excluded from the present study based on self report data.

Methods for the ongoing longitudinal study have been previously described (17). In short, bi-annual research visits are scheduled at which time standardized interviews are administered (including drug use and medical histories), physical examinations and phlebotomy are performed. Laboratory analysis includes HIV-1 antibody testing by enzyme immunoassay (EIA) with confirmatory testing by Western Blot (Abbott Laboratories, North Chicago, Illinois; DuPont, Wilmington Delaware, USA). Complete blood counts and lymphocyte subset analyses are performed at the New York Blood Center (New York City, New York, USA).

Data collected as part of the ongoing longitudinal study and used for the present analysis included $CD4^+$ and $CD8^+$ cell counts per mm^3, hemoglobin (hgb) levels, drug use (heroin, cocaine, alcohol) and injection history in the six months prior to the date of the blood specimen, methadone dose, age, zidovudine use, height and weight at the time of the blood specimen and clinical disease status. Symptomatic status is defined as diarrhea or fever

for greater than two weeks or unintentional weight loss (not attributed to drug use) of more than five pounds. AIDS was defined according to the 1987 Centers for Disease Control definition (18). Body mass index (BMI) was calculated (Quetelet)(19) as weight [kilograms]/height^2 [meters] and converted to BMI percentiles by age for males and females using standard anthropometric charts (20). Serum specimens from the subjects' most recent visit stored at -70°C were retrieved for vitamin A measurements. Serum vitamin A levels were measured using high-performance liquid chromatography (21), and quality control was monitored with vitamin A reference standards from the National Institute of Standards and Technology (Gaithersburg, Md, USA).

Vitamin A levels were categorized similarly to other studies and were as follows: <0.70 μmol/L, 0.70-1.04 μmol/L, 1.05-1.39 μmol/L and ≥ 1.40 μ mol/L (16). Deficiency was defined at <1.05 μmol/L and severe deficiency at <0.70 μmol/L (22). Low hgb was defined as <13.5/ dL for males and <12.5 g/dL for females. $CD4^+$ cell count was categorized as <200, 200-500 and >500 cells/mm^3. As previous work in our cohort found $CD4^+$ cell counts of <150 cells/mm^3 to be prognostic of AIDS defining clinical conditions(23), we examined the association of vitamin A levels and $CD4^+$ cell count using both $CD4^+$ cell count above and below 200 and above and below 150 cells/mm^3.

Data analysis was performed using SAS (SAS Institute, Inc. Cary, North Carolina USA). Univariate tests of association were performed using chi square or Fisher's exact test (24). Multivariate logistic regression was used to evaluate the association of vitamin A and anemia and to examine vitamin A deficiency as the dependent variable. Interaction and confounding effects were evaluated (24).

Modeling was accomplished by backwards stepwise regression. Variables selected for the final model were based on the likelihood ratio statistic (LRS), i.e., twice the change in the model likelihood. Criteria for inclusion in the final model was a LRS with a significant chi-square for the corresponding degrees of freedom i.e., ≥ 3.8 for 1 d f, or a change in the OR of variables in the model of more than 10%. The adjusted odds ratios (OR_{adj}) are presented with the corresponding 95% confidence intervals (95% CI). All tests were two tailed with $\alpha = 0.05$.

RESULTS

A total of 135 HIV-infected and 45 HIV-uninfected IDUs were evaluated. Table 1 presents the distribution by HIV serostatus for demographic, drug history and clinical characteristics. A significantly lower proportion of the seropositive subjects were females. A significantly greater proportion of the seropositive subjects used cocaine while there was no difference in alcohol or injection use.

No subject reported a history of chronic fever during the six months prior to and including the time of blood drawing. To assess whether methadone was affecting serum vitamin A levels, we examined the relationship between serum vitamin A and methadone dose. There was no evidence of a relationship between vitamin A levels and methadone dose and no difference in vitamin A deficiency or median vitamin A levels between the lowest and highest methadone dose categories (data not presented).

Serum vitamin A levels did not differ significantly by HIV serostatus. At least one

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third of both the HIV seropositive and HIV seronegative subjects had vitamin A deficiency (<1.05μmol/L). Seventeen percent of the HIV seropositive subjects and 11% of the HIV seronegative subjects had severe vitamin A deficiency (<0.70 μmol/L). (See table 1)

Table 2 shows the percentage of vitamin A deficient individuals by HIV serostatus, for demographic, drug history and clinical characteristics. Among HIV seropositive subjects, females were significantly more likely to be vitamin A deficient (p=0.05); median vitamin A levels were 1.11 μmol/L for women and 1.29 μmol/L for men, p=0.06. There was no difference in vitamin A levels between those subjects with <200 and ≥200 cells/mm^3. Median serum vitamin A was significantly lower for those with CD4$^+$ <150 cells/mm^3 compared to those with CD4$^+$ ≥150 cells/mm^3, a level previously found to be prognostic in our cohort (0.81 μmol/L versus 1.19 μmol/L for <150 and ≥150 cells/mm respectively, p=0.02). Vitamin A deficiency was present among 37% of asymptomatic patients, 28% of symptomatic patients, and 30% of AIDS patients. Mean hgb was significantly lower in the vitamin A deficient individuals(12.4 g/dL versus 13.0 g/dL respectively, p=0.04). Figure 1 shows the relationship between vitamin A levels and low hgb among the HIV seropositive subjects. Among those with the lowest vitamin A levels, 68% were anemic compared to 41% in the highest vitamin A category.

Among HIV-seronegative subjects, females also tended to be more vitamin A deficient (p=0.08). Vitamin A levels were lower, albeit not significantly so, for HIV seronegative women compared to men (median vitamin A of 1.03 μmol/L for women compared to 1.27 μmol/L for men, p=0.25). Fifty -seven percent of the HIV seronegative women had vitamin A deficiency compared to 29% of the men. The lack of statistical significance is likely due to the small sample size and inadequate power. Vitamin A deficiency was not associated with low hgb among the HIV seronegative individuals.

Multivariate

Among the HIV seropositive individuals female gender was independently associated with vitamin A deficiency after controlling for BMI, CD4$^+$ cell count and age (OR_{adj} 1.84; 95% CI 1.16-2.93). Table 3 presents the OR_{adj} and 95% CIs for the final model. Results were similar using CD4$^+$ cell count categorized as <150 and ≥150 cells/mm^3 or CD4$^+$ categories (<200,200-499 and ≥500 cells/m^3. Among the HIV seronegative subjects, a multivariate analysis that included BMI, drug history and age revealed no statistically significant independent associations with vitamin A deficiency (data not shown). There were too few women to include gender in the analysis.

DISCUSSION

In the present study, 35% of the HIV seropositive subjects and 33% of the HIV seronegative subjects had vitamin A deficiency. The prevalence of vitamin A deficiency in our HIV-infected subjects is notably higher than the 15% reported by Semba et al in another sample of HIV-infected IDUs in Baltimore, Maryland (16). Other studies in HIV-infected individuals with diverse risk behaviors have reported the prevalence of vitamin A deficiency ranging from 11-29% (11-15). The higher rates were reported in study samples consisting of patients with more advanced stages of HIV disease, those with AIDS or AIDS related complex (13). One study that included HIV seronegative homosexuals found 0% were

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vitamin A deficient (11). Our study suggests that IDUs in the Bronx, New York City have among the highest prevalence of vitamin A deficiency reported in adult population based studies in the United States to date. Severe vitamin A deficiency (<0.70 μmol/L) was present in 17% of our HIV seropositive subjects, a prevalence comparable to the 22% reported by Karter et al in hospitalized AIDS patients (25). As only 30% of our subjects

TABLE 1
Distribution by Serostatus for Demographic, Drug History , Clinical Characteristics and Vitamin A Levels Among HIV-Infected Injecting Drug Users

Variable	N(Percent) HIV+	HIV-	p value *
Race/ethnicity			
White	15(11)	9(20)	
Black	12(9)	6(13)	
Hispanic	108(80)	30(67)	ns
Age Group#			
<35 yrs	68(51)	25(56)	
≥35 yrs	65(49)	20(44)	ns
Gender			
Female	43(32)	7(16)	
Male	92(68)	38(84)	.04
Methadone Dose#			
<60 mg	57(42)	15(33)	
≥ 60 mg	76(57)	30(66)	ns
Low hemoglobin**	69(51)	12(27)	.004
Serum Vitamin A			
<0.70 μmol/L	23(17)	5(11)	
0.70-1.04 μmol/L	24(18)	10(22)	
1.05-1.39 μmol/L	39(29)	15(33)	
≥1.40 μmol/L	49(36)	15(34)	ns
Body Mass Index			
<25th percentile	67(50)	26(58)	
25-50th percentile	26(19)	5(11)	
>50th percentile	42(31)	14(31)	ns
Alcohol use (prior 6 mos.)	38(28)	11(24)	ns
Injection use (prior 6 mos)	43(32)	12(27)	ns
Cocaine use (prior 6 mos)	66(49)	9(20)	.001
Cocaine or crack use (prior year)	76(56)	16(36)	.02
$CD4^+$ cell count			
<200 cells/mm³	47(35)	0	
200-500 cells/mm³	48(36)	0	
≥500 cells/mm³	40(30)	45(100)	<.001

* Chi-square and Fisher's exact test
# 2 subjects are missing data
**Low hemoglobin: <13.5 g/dL males; <12.5 g/dL females

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TABLE 2
Proportion with Serum Vitamin A Deficiency (<1.05 μmol/L) by Serostatus for Demographic, Drug History and Clinical Characteristics Among Injecting Drug Users

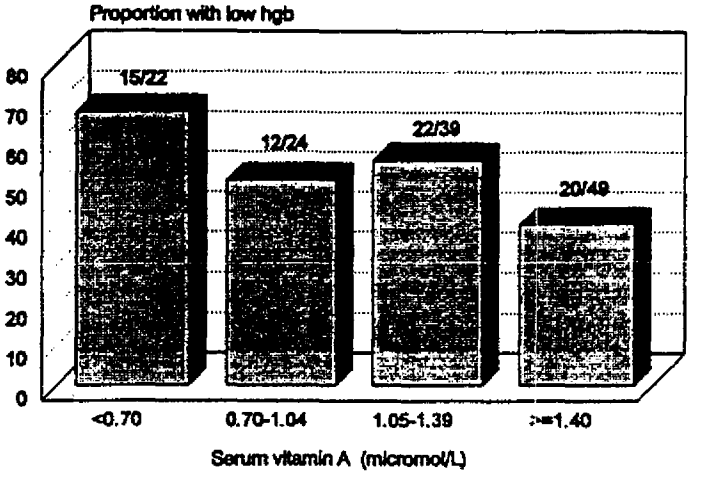
Variable+	n/N (Percent) HIV+	HIV-
Race/ethnicity		
White	4/15 (27)	3/9 (33)
Black	6/12 (50)	2/6 (33)
Hispanic	37/108 (35)	10/30 (33)
Age Group		
<35 yrs	23/68 (34)	8/25 (32)
≥35 yrs	24/65 (37)	7/20 (35)
Gender*		
Female	20/43 (46)	4/7 (57)
Male	27/65 (29)	11/38 (29)
Body Mass Index		
<25th percentile	25/67 (37)	6/26 (23)
25-50th percentile	9/26 (35)	2/5 (40)
>50th percentile	13/42 (31)	7/14 (50)
Alcohol use(previous 6 mos.)		
Yes	13/38 (34)	3/11 (27)
No	34/97 (35)	12/34 (35)
Injection use(previous 6 mos.)		
Yes	13/43 (30)	4/12 (33)
No	34/92 (37)	11/33 (33)
Cocaine use(previous 6 mos.)		
Yes	25/66 (38)	1/9 (11)
No	22/69 (32)	14/36 (32)
Cocaine or crack use (previous year)		
Yes	29/76 (38)	4/16 (25)
No	18/59 (31)	11/29 (38)

*Females were more likely to be vitamin A deficient; p=0.05 for HIV seropositive and p=0.08 for HIV negatives.

were symptomatic or had AIDS defining clinical conditions at the time of the serum determination, these data suggest that a high proportion of HIV-infected subjects in our cohort may be vitamin A-deficient before the onset of clinical symptoms. Similar to previous studies, women in this study population were more likely to be vitamin A deficient (1).

An acute phase response is unlikely to be responsible for the vitamin A deficiency in this cohort as both HIV infected and uninfected IDUs had high levels of vitamin A deficiency and there were no reported infections at the time or in the six months prior to the vitamin A determination. This study also found no evidence to suggest a relationship between specific

FIGURE 1
Serum Vitamin A and Low Hemoglobin Among HIV Seropositive Injecting Drug Users



Corresponding number of subjects appears above each bar.
Low hemoglobin: < 13.5 g/dL for males; < 12.5 g/dL for females.
Mantel Haenszel trend test, p=0.05.

TABLE 3
Adjusted Odds Ratios (OR_{adj}) and 95% Confidence Intervals (95% CI) of Logistic Regression for Vitamin A Deficiency Among HIV-Infected Injecting Drug Users

Variable	OR_{adj}	(95% CI)
Gender		
Men	1.00	-
Women	1.84	(1.16-2.93)
Body Mass Index		
>50th percentile	1.00	-
25th-50th percentile	1.57	(0.52-4.75)
<25th percentile	2.44	(0.90-6.72)
CD4+ cell count		
>500 cells/mm^3	1.00	-
200-500 cells/mm^3	0.67	(0.25-1.80)
<200 cells/mm^3	0.81	(0.32-2.04)
Age Group		
<35 years	1.00	-
≥35 years	1.46	(0.66-3.22)

illicit drug use and vitamin deficiency. In this population, which is predominantly Hispanic,

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inadequate dietary intake is the most likely explanation for the notably high proportion of individuals with vitamin A deficiency. Other biological mechanisms contributing to low vitamin A levels may include high, abnormal urinary losses of vitamin A during HIV infection (26) and general hepatic depletion of vitamin A, which has been well described with infections (27).

The importance of vitamin A lies in its role in immunity to infectious diseases which is exemplified by the well recognized relationship between vitamin A and infectious disease morbidity and mortality. Vitamin A is an immune enhancer and an essential co-factor in T and B-cell activation and growth (2,38-30). It also plays a major role in the maintenance of mucosal epithelium (31) and is necessary for normal differentiation of hematopoietic stem cells (32). Deficiency of vitamin A is characterized by a loss of $CD4^+$ cells (8,9) and is associated with impaired hematopoiesis and anemia (33). Vitamin A supplementation trials in clinically and subclinically deficient children have been associated with restored immune function, reduction in anemia, and reduced infectious disease morbidity and mortality (2,34-36). The biological basis for the reduction in morbidity and mortality by vitamin A appears to be immunopotentiation, which has been demonstrated in both animals and humans with normal and deficient vitamin A status (2,28-30,37).

In the present study, median vitamin A levels among seropositive subjects were significantly associated with $CD4^+$ cell count levels previously found to be prognostic of HIV disease progression in our cohort (<150 versus ≥ 150 cells/mm). These findings corroborate previous findings of vitamin A levels and $CD4^+$ cell count in HIV infected IDUs (16). Of particular interest, is the fact that vitamin A deficiency, while marked in both HIV seropositive and seronegative subjects in our study, was associated with low hemoglobin only among the HIV seropositive subjects. While the association of vitamin A deficiency and low hemoglobin is consistent with other data (16,36,38), our finding of this association only in the HIV seropositive subjects may suggest an interaction between low vitamin A and HIV infection. Vitamin A deficiency may contribute to nutritional anemia through impaired iron mobilization and the resulting decrease in iron available for red cell formation (38).

Injecting drug users represent an increasing proportion of the HIV-infected population. As drug use is generally associated with lower socio-economic status these individuals may be at greater risk of micronutrient deficiency. A carefully designed study to look prospectively at the relationship between vitamin A deficiency and infectious disease morbidity in HIV- infected and uninfected high risk IDUs may be warranted, particularly given the high prevalence of vitamin A deficiency observed in our cohort. It is possible that the marked degree of undernutrition and potential metabolic alterations associated with drug use itself may promote vitamin A deficiency. Intake of fat, zinc and protein are necessary for vitamin A absorption and utilization (39). Co-existing deficiencies in these nutrients may also impact on vitamin A levels in HIV-infected individuals. Studies of specific micronutrients and HIV disease progression suggest an association with vitamin A deficiency along with other micronutrients(40-42). Among HIV-infected IDUs serum retinol levels were associated with lower circulating $CD4^+$ T-cells and higher mortality (16,42). Among HIV-infected homosexuals a higher intake of vitamin A was associated with increased $CD4^+$ T lymphocyte count (40) and moderate intakes were associated with slower progression to AIDS(41). A more complete characterization of micronutrient deficiencies among IDUs is needed as micronutrient supplementation might provide an inexpensive, low cost intervention to reduce disease morbidity and mortality among HIV-infected and uninfected high risk IDUs.

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Sampling Designs for Xerophthalmia Prevalence Surveys

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Katz J (Department of International Health, Johns Hopkins School of Hygiene and Public Health, Room 5515, 615 N Wolfe Street, Baltimore, MD 21205–2103, USA), Yoon S S, Brendel K and West K P Jr. Sampling designs for xerophthalmia prevalence surveys. *International Journal of Epidemiology* 1997; **26:** 1041–1048.

Background. The purpose of this study was to estimate the bias and design effects associated with the Expanded Program on Immunization's (EPI) sampling design when estimating xerophthalmia prevalence, and to estimate the savings associated with EPI in terms of distance travelled within selected clusters.

Methods. Computer simulation of the EPI sampling strategy was done using maps from a xerophthalmia survey of 40 wards in Sarlahi district, Nepal. Samples of fixed cluster sizes of 7, 10, 15, 20 and 25 were compared. The estimated prevalence using the EPI design was compared with the true prevalence in the 40 wards to estimate the bias. The design effect was estimated by taking the ratio of the variance under EPI sampling to that of stratified random sampling (SRS) with fixed cluster sizes. The EPI was also modified by increasing the distance between selected houses from nearest neighbour to skipping 1–4 houses between selected ones. The difference between the distance travelled within clusters using SRS compared with EPI was weighed against the bias and increased variance.

Results. The prevalence of xerophthalmia was 2.8%. The EPI design overestimated xerophthalmia prevalence by between 0.27% and 1.16%. The design effects of EPI cluster sampling within wards varied between 0.73 and 1.35. Neither the bias nor the design effect was related to distance between households or cluster size. Distance travelled within wards was always less for EPI designs with cluster sizes of 7 or 10. There was no saving in terms of distance travelled for designs with cluster sizes from 15 to 25 if there were two or more houses between selected ones. For fixed cluster sizes of 15 or fewer, the EPI sampling design using nearest or next nearest neighbours is a better choice than SRS in terms of minimizing the distance travelled and the mean square error.

Conclusions. The choice of an optimum method would need to account for the density of clusters and difficulty of travel between clusters, as well as the costs of travel within clusters. Based on certain assumptions, EPI with 15 children per cluster would be favoured over examining all children in selected wards unless the travel time between wards was more than 2 days.

Keywords: sampling, survey, vitamin A deficiency, xerophthalmia, Nepal

Xerophthalmia is the leading cause of childhood blindness worldwide.[1-3] Children with xerophthalmia have been shown to be at increased risk of mortality,[4] morbidity,[5-7] and poor growth.[8] In areas where xerophthalmia is a public health problem, vitamin A supplementation with capsules or through fortification can reduce mortality by an average of 23% and avert between one and two million deaths annually among children aged 1–4 years.[9,10] A number of existing national vitamin A supplementation programmes have been given renewed urgency by these findings, while new national programmes are beginning in other countries. Although xerophthalmia is only the tip of the iceberg in terms of underlying subclinical vitamin A deficiency, it is a relatively easy and non-invasive way to assess whether vitamin A deficiency is a public health problem. Furthermore, a decline in xerophthalmia prevalence is one way to measure the impact of programmes. A xerophthalmia prevalence survey can be done to assess whether a problem exists. Such a survey can also act as a baseline from which to judge the progress of programmes through a comparison with follow-up surveys. For these purposes, ways in which to reduce the complexity, cost and time taken to complete surveys would be useful.

The Expanded Programme on Immunization (EPI) of the World Health Organization (WHO) advocates a sampling procedure for surveys of immunization coverage that does not require mapping or a census of selected clusters.[11-19] The purpose of this procedure is to make it simple enough to be used by health workers with minimal technical support in field settings. The approach has been adapted for surveys of many other health outcomes such as nutritional status and for morbidity surveys.[20,21] However, EPI has the potential

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to lead to increased variance and bias of the coverage or disease prevalence estimates.[22–25] In this study, we use maps drawn for a baseline xerophthalmia survey in Nepal to simulate stratified random sampling (SRS) and EPI sampling of this population so as to obtain estimates of the bias and increased variance associated with EPI sampling for xerophthalmia surveys. These estimates are then used, in conjunction with the difference between the distance travelled within clusters using EPI and SRS, to make recommendations about the optimal sampling design for xerophthalmia surveys.

MATERIALS AND METHODS

Source of Data

The data used in this study come from a baseline survey of xerophthalmia undertaken prior to a randomized community trial of vitamin A supplementation in Sarlahi district, Nepal.[26] Forty wards in 25 Village Development Committees were selected at random from the larger sample of 261 wards in 29 Village Development Committees that were included in the trial. A ward is a well defined administrative unit, with nine wards comprising each Village Development Committee. The mean number of households per ward was 72.7. The mean number of children per ward was 119.1, and the mean number of children per household was 1.6. Within neighbourhoods, houses are relatively close (5–10 metres on average), but there are often several neighbourhoods within wards that can be spaced up to half a kilometre apart. Sarlahi district is located in the lowland terai area bordering Bihar, and was selected because it was identified as an area with high rates of xerophthalmia in two previous national surveys.[27,28] The selected wards were mapped, all houses were numbered, and children under 5 years old were censused by interviewers who visited each household. Parents were invited to bring eligible children to a central site in the ward for an eye examination. Parents were asked about night blindness using local terms. An ophthalmologist or senior ophthalmic assistant examined the eyes for signs of xerophthalmia which were graded according to the WHO definition.[29] Prior inter-observer trials with 1001 children showed excellent agreement between the two graders, with a kappa statistic of 0.93.

Sampling Designs

The comparison of all sampling designs assumed that the primary sampling units (wards) had already been selected proportionate to ward size, and that a fixed number of children would then be selected in varying ways from each ward. The first design selected a fixed number of children at random from each of the 40 wards, thereby guaranteeing equal probability of selection within each ward. Other simulations used the EPI cluster design or modifications of this design to select a fixed number of children per ward. The EPI cluster sampling method is described in detail in the WHO manual.[11] Briefly, the interviewer goes to the centre of the village and selects a direction at random (spinning a bottle has been suggested). The houses along this direction are numbered from the centre to the periphery and one is chosen at random to be the starting house in the sample. All children of eligible age are identified in this house and immunization coverage information is obtained. The interviewer then goes to the next nearest house to the right of the front door of the starting house. If a house has no eligible children, the next nearest house is visited. This continues until the immunization status of at least seven children has been determined. All children in selected houses are surveyed so that a cluster may contain more than seven children.

Simulation of EPI Sampling

The maps of the 40 wards were digitized and the x-y coordinates of each house were stored in computerized format with the associated identifiers of the preschool children in that house. The coordinates were selected relative to some arbitrary origin. The coordinates of all the households were then averaged to identify an approximate centre of each ward. This location was then set as the origin and the household coordinates were adjusted to reflect this origin. A direction was chosen at random from the origin. A pair of parallel straight lines of a fixed width was drawn from the centre to the periphery. This was done because houses in the computerized database were dimensionless locations, and unless a path of a certain width was drawn, the likelihood of a house falling exactly on the line would be very low. After the starting house was selected, the next nearest neighbour from +90° to −90° was selected. This was done in order to simulate the EPI rule of going to the next nearest house to the right when facing North. Once the edge of the village was reached, the direction of search was rotated 90° to the right and selected the next nearest house on this path. If the selected house had already been visited or if the house did not have any eligible children, the next nearest house to its right was visited. Since xerophthalmia is a much rarer outcome than positive immunization status, fixed cluster sizes within each ward were varied to include 7, 10, 15, 20 and 25 children selected per ward by either SRS or EPI cluster sampling. If the ward had fewer children than the requisite number per ward, then all children in that ward were included. Only three wards had fewer than 25 children. Modifications to the EPI approach increased

the distance of households from nearest neighbour to the selection of every second, third, fourth and fifth house to the right of each selected house.

Estimation of Prevalence and Design Effects

The true prevalence was estimated by dividing the number of cases of xerophthalmia in the 40 wards by the total number of children examined. In an actual survey in which the primary sampling units (wards) were selected proportionate to size, the sample would be self-weighting. However, in this simulated study, there has been no selection proportionate to size, so the comparison of interest is between the 'true' prevalence in the 40 wards and the estimated prevalence based on sampling a fixed number of children in each of the 40 wards. Therefore the prevalence of xerophthalmia for each simulation sample was calculated by taking the average of the ward prevalences weighted by the ward size. One thousand simulated samples were drawn for each sampling design and each sample size. The mean prevalence and the empiric variance of the 1000 samples were calculated for each sampling design (variance $= \Sigma (p_i - p)^2/999$ where p_i is the prevalence in the ith ward and p is the average prevalence across the 1000 simulations). The bias of the EPI design was estimated by taking the difference between the 'true' prevalence in the 40 wards and the mean prevalence of the 1000 samples selected according to EPI cluster sampling.

The design effect was calculated by taking the ratio of the variance from EPI sampling to that of stratified random selection of a fixed sample size per ward. This design effect is a measure of the extra variation generated by sampling a cluster or local neighbourhood of houses within a ward rather than sampling children at random within wards, where children would tend to be scattered across the ward. The mean square error associated with EPI sampling was calculated by taking the variance of EPI sampling plus the square of the bias. The mean square error of SRS was estimated by taking the variance under simple random sampling, since there should be no bias for this sampling design.

Estimation and Comparison of Distance Travelled

For EPI sampling, the simulated surveyor started at the centre of the ward, travelled in a straight line to the first selected house, and then travelled sequentially to each next nearest house to the right. The total distance travelled in the ward was calculated by adding the straight line distances along the path just described. This included visits to houses where no eligible children resided. For simple random sampling, the distance was calculated by taking the shortest straight line path from the first randomly selected house to the next nearest house and from there to the next nearest house until all randomly selected houses had been visited. This excluded visits to houses without children because they were not included in the sampling frame. Because the maps of each ward were not drawn to the same scale, the ratio of the total distance travelled for the 1000 simulated EPI samples to that of SRS was calculated separately for each ward. The median of the ward ratios was used to compare the overall distances travelled using EPI sampling to that using SRS. The median was chosen because the distribution of the distance ratios was skewed to the right.

To compare sampling designs in terms of the trade-off between distance travelled and mean square error, the EPI mean square error multiplied by the median distance travelled under EPI sampling was divided by the SRS mean square error multiplied by the distance travelled under SRS. This assumes the cost of doubling the mean square error is the same as doubling the time. If this ratio was less than one then EPI would be favoured over SRS in terms of less distance travelled for comparable mean square error. In order to evaluate designs in terms of increased variance alone, the ratios of the EPI variance multiplied by distance to that of SRS variance multiplied by SRS distance was calculated. If this ratio was less than one, the EPI approach would be favoured over SRS in terms of less distance travelled for comparable variance.[30]

An alternative to cluster sampling using EPI or SRS would be to examine all children in selected villages, since the cost of travel to villages is greater than the cost of travel within villages. The cost of these different survey designs can be calculated in days to complete the survey using the following formula: $mn/x + mt_1 [(n/y) - 1] + mt_2$ where m is the number of clusters, n is the number of children examined per cluster, x is the number of children that can be examined per day, y is the number of children per household, t_1 is the average travel time between houses in days, and t_2 is the average travel time between villages in days. The total sample size, $N = nm$, varies according to the design effect of cluster sampling, which is greater the larger the cluster size.[31,32]

RESULTS

A total of 4920 households were censused in the 40 wards, and 57.4% of households were identified as having children under 5 years of age. The number of children under 5 living in these households was 4765. Of these, 4297 (90.2%) were interviewed about their morbidity in the past week and examined for xerophthalmia. These children formed the population from

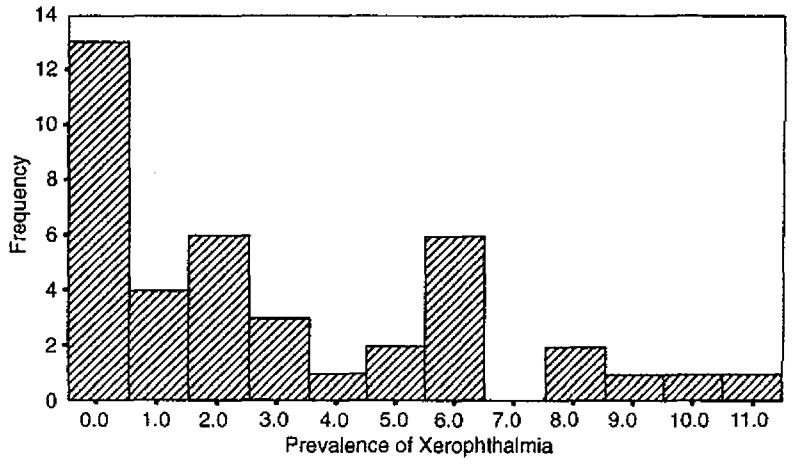

FIGURE 1 *Frequency distribution of xerophthalmia prevalence by ward, Nepal, 1989*

TABLE 1 *Design effect at the second stage of cluster sampling ($Variance_{EPI}$ / $Variance_{SRS}$), Nepal, 1989*

Cluster size	No. of houses skipped				
	0	1	2	3	4
7	1.11	0.90	1.12	1.20	1.16
10	1.19	0.91	1.11	1.35	1.15
15	1.13	1.15	1.00	1.10	1.15
20	1.20	1.00	0.80	0.93	0.83
25	0.90	0.80	0.73	0.83	0.83

TABLE 2 *Bias (sample prevalence minus true prevalence in per cent), Nepal, 1989*

Cluster size	No. of houses skipped				
	0	1	2	3	4
7	0.49	0.27	0.77	0.86	0.95
10	0.63	0.30	1.00	0.92	1.16
15	0.74	0.55	1.10	0.64	0.94
20	1.04	0.79	0.98	0.57	0.70
25	1.00	0.75	0.79	0.54	0.92

which repeat samples were drawn. The number of children in each ward ranged from 13 to 284 and the number of households ranged from 31 to 315. The prevalence of xerophthalmia was 2.8% among the 4297 children surveyed in the 40 wards. However, the ward prevalences ranged from 0% to 11% (Figure 1). The design effect at the second stage of sampling (variance of EPI divided by the variance of SRS of a fixed number of children per ward) ranged from 0.73 to 1.35 (Table 1).

The bias generated by EPI sampling ranged from 0.27% to 1.16% (Table 2). The EPI design consistently led to overestimation of xerophthalmia prevalence. The true prevalence was 2.8% but the EPI prevalences ranged from 3.1% to 4.0% (Figure 2). The bias was not related to sample size per cluster, nor to number of houses between selected houses.

As expected, the median distance ratios for EPI relative to SRS increased with distance between houses and with the increase in fixed cluster size (Table 3). The larger the cluster size, the greater the proportionate increase in the distance ratio. For next nearest houses, the distance travelled was always less for EPI than SRS, even when sampling 25 children per cluster. However, for houses with at least two houses between them and for cluster sizes of 20 or more, the advantage of EPI in terms of distance travelled disappeared. For houses with four houses between them, EPI offered a clear advantage for cluster sizes of seven only. Based on minimizing the combination of mean square error and distance travelled, EPI was preferable for cluster sizes of 10 or fewer and for houses spaced close together (Table 5). Based on minimizing the combination of variance and distance travelled, EPI was preferred over SRS for all cluster sizes where no more than two houses were between selected houses (Table 6).

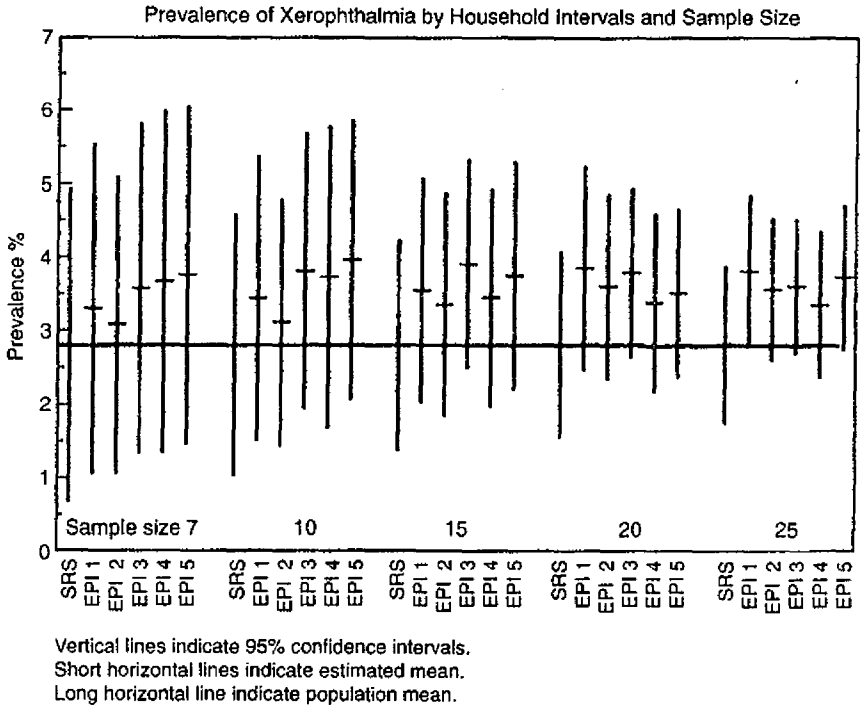

FIGURE 2 *Prevalence and 95% confidence intervals for xerophthalmia using different sampling designs, Nepal, 1989*

TABLE 3 *Median ratios of distance[a] travelled within wards for Expanded Program on Immunization (EPI) versus Stratified Random Sampling (SRS), Nepal, 1989*

Cluster size	No. of houses skipped				
	0	1	2	3	4
7	**0.39**	**0.53**	**0.64**	**0.72**	**0.82**
10	**0.42**	**0.58**	**0.68**	**0.80**	**0.92**
15	**0.49**	**0.62**	**0.80**	1.01	1.07
20	**0.55**	**0.77**	**0.92**	1.28	1.33
25	**0.65**	**0.95**	1.13	1.47	1.62

[a] Ratio of <1 means EPI favoured over SRS.

TABLE 4 *Ratios of mean square error for Expanded Program on Immunization (EPI) to mean standard error for Stratified Random Sampling (SRS), Nepal, 1989*

Cluster size	No. of houses skipped				
	0	1	2	3	4
7	1.31	1.02	1.63	1.83	1.93
10	1.69	1.02	2.37	2.41	2.84
15	2.18	1.73	3.33	1.88	2.85
20	3.84	2.51	3.17	1.71	2.02
25	4.23	2.66	2.80	1.80	3.67

Given the above results, EPI appears to be a good alternative to SRS at the second stage of sampling (at the ward level) since the cost in terms of distance travelled within wards was much lower for EPI using nearest neighbours. However, the estimates of prevalence generated by EPI for cluster sizes from seven to 25 were overestimated by between 11% and 36%. In addition, other important costs come into play in the choice of design. The time it takes to travel from one cluster to another may make it more efficient to survey every child in the cluster. One disadvantage of this latter design is that the design effect increases with the size of the cluster. Figure 3 gives the time taken in days to complete a survey using EPI with fixed cluster sizes

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TABLE 5 *Efficiency[a] of Expanded Program on Immunization (EPI) relative to Stratified Random Sampling (SRS) = (median distance$_{EPI}$ × Mean Square Error $[MSE]_{EPI}$)/(median distance$_{SRS}$ × MSE_{SRS}) by cluster size and house proximity, Nepal, 1989*

Cluster size	No. of houses skipped				
	0	1	2	3	4
7	**0.51**	**0.51**	1.04	1.32	1.58
10	**0.71**	**0.59**	1.61	1.93	2.61
15	1.07	1.07	2.66	1.90	3.05
20	2.11	1.93	2.92	2.19	2.69
25	2.75	2.53	3.16	2.65	5.94

[a] Ratio of <1 means EPI favoured over SRS.

TABLE 6 *Median distance ratios of Expanded Program on Immunization (EPI) to Stratified Random Sampling (SRS) multiplied by the design effect[a] by cluster size and house proximity, Nepal, 1989*

Cluster size	No. of houses skipped				
	0	1	2	3	4
7	**0.43**	**0.48**	**0.72**	**0.86**	**0.95**
10	**0.50**	**0.53**	**0.75**	1.08	1.06
15	**0.55**	**0.71**	**0.80**	1.11	1.23
20	**0.66**	**0.77**	**0.74**	1.19	1.10
25	**0.59**	**0.76**	**0.82**	1.22	1.34

[a] Ratio of <1 means EPI favoured over SRS.

of 10 or 15, compared with one in which all children in selected wards are examined. Sample size calculations indicate that approximately 1000 children need to be examined in order to estimate a prevalence of 2.8% (95% confidence interval : 1.8–3.8). This is the sample size needed if a truly simple random sample were selected from a list of all children in the population of interest. However, the design effect using EPI with clusters of size 10 is about 1.4, and about 1.5 for clusters of size 15. Hence, 1400 or 1500 children must be examined in 140 or 100 wards, respectively. If all children in selected wards are to be examined, then approximately 5500 children in 55 wards are needed because the design effect is 5.5 for clusters of size 100 (the average number of children per ward).[31] The calculation assumes that the travel time between houses is

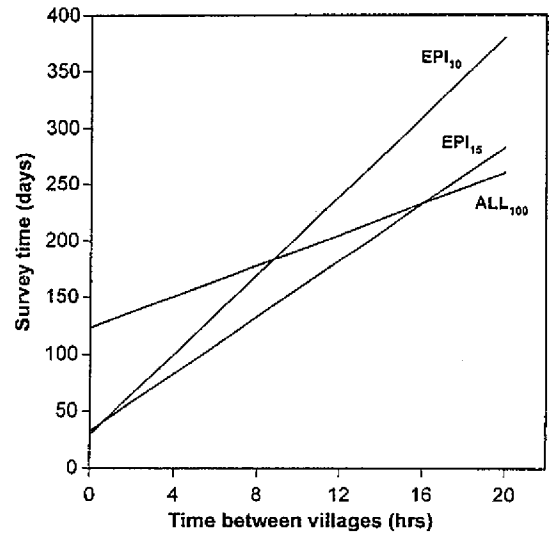

FIGURE 3 *Total time to complete a survey plotted against the average travel time between clusters for the Expanded Program on Immunization (EPI) with cluster size 10 and 15, and for a design that examines all children in selected wards, Nepal, 1989*

5 min, that there are 1.8 children per household who are under 5 years and that 8 h of work can be done in one day. If 60 children can be examined in 8 h, then EPI with 10 children per cluster takes less time than examining all children in selected wards, unless the travel time between wards is more than 8 h and 45 min. If 100 clusters of 15 children are examined, then the break point is about 2 days travel time between wards. Variations in the travel time between houses (t_1) changes the intercept but not the slope of the cost lines. An increase in the travel time between houses would reduce the travel time between wards where EPI and 'all children examined' designs were comparable in terms of time costs. A decrease in t_1 would have the opposite effect.

DISCUSSION

Less expensive and more rapid ways of estimating the prevalence of xerophthalmia would be very helpful for population assessment and surveillance. Because of the logistics and cost of constructing a sampling frame of all children in a population, simple random sampling from the entire population is almost never an option. A sampling frame of communities such as villages, wards, or city blocks can often be constructed with estimates of community size based on census information already

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available. However, variations in how to sample individuals from communities that have been selected proportionate to size can substantially alter the accuracy, speed and cost of surveys. The EPI sampling design reduces the cost of surveys by removing the need to map and census selected communities, and by reducing the time taken to travel within communities. This reduced cost needs to be balanced against the potential bias and increased variance generated by such a sampling design. To date, no estimates of the bias and increased variance have been available for xerophthalmia surveys where EPI sampling is used. The magnitude of the estimate of xerophthalmia prevalence in this population is comparable to other populations in which xerophthalmia is of public health importance; 3.9% in Malawi, 1.3% in Zambia, 1.9% in Indonesia.[31]

The magnitude of the bias and increased variance will depend on the geographical distribution of xerophthalmia within communities. If cases of xerophthalmia are evenly spread across the community, then there should be no bias or increased variance from EPI sampling. Increased variance in the absence of bias can result if clusters of cases are found in certain neighbourhoods. The selection of a cluster of households in a neighbourhood (as tends to be the case with EPI sampling) will produce prevalence estimates that are sometimes too low, and sometimes too high, but on average the prevalence will be accurately estimated. Bias arises if there is a systematic pattern of distribution of cases in the community, and if the starting household in the EPI design is not selected at random. A consistent overestimate of prevalence using EPI, as seen in these data, might arise if cases were concentrated in the centre of the ward and if EPI more often resulted in the selection of a starting house near the centre. This was not a likely explanation because, when the ward was divided into an inner and outer circle, one of which was selected at random to contain the starting house, the bias was not reduced. The bias was also not reduced by increasing the distance between surveyed houses, thereby spreading the sample across a larger area of the ward. The bias was smaller when cluster sizes were small and there were fewer houses between selected houses. These findings are contrary to those of Bennett for other health outcomes.[22] However, we found similar results to Bennett for different morbidity outcomes such as diarrhoea, so the bias pattern appears to vary with the health outcome of interest. In this population, the variance was increased by using EPI, but only for small cluster sizes and houses of close proximity, and the largest design effect would have resulted in a 35% increase in variance of the prevalence estimate (a 16% increase in the length of the confidence interval).

Obviously, a design in which fewer communities are visited but more children within the communities are examined is preferred because of the cost of travelling between communities. However, the more children examined per community, the larger the design effect and the greater the total number of children who need to be examined. For example, the design effect was 5.5 if all children in the 40 wards were examined.[31] This would result in the need to examine 5500 children in 55 wards (100 children per ward) to obtain a 95% confidence interval around the prevalence estimate of 1.8–3.8. To achieve the same level of precision, a design that sampled 15 children per ward would require only 1500 children but necessitate visiting 100 wards. Hence, the distance between communities is important in deciding which design is less costly. Other costs include reimbursement of trained examiners (the same per unit time for both designs) and the cost of mapping and census-taking (for SRS and 'all children examined' designs). In our study, the average ward took 3 days to map and census. The cost of trained examiners as a per cent of total costs is relatively small, but would vary by whether a local or expatriate ophthalmologist, or ophthalmic assistant was hired. In the context of our data, EPI with 15 children per cluster would be favoured over examining all children in a ward if travel time between wards did not exceed 2 days on average. With clusters of size 10, EPI would be favoured if time between wards did not exceed 8 h. If fewer children can be examined per day, if the distance between houses within communities is greater, or if there are fewer eligible children per household, then examining all children will become preferable with a shorter travel time between communities. While these calculations provide guidelines for the choice of sampling design, the situations may vary from one population to another. The design effect when sampling all children in selected villages ranged from 2.03 to 5.50 for two African and two Asian countries with a median number of children per cluster ranging from 25 to 99.[31] Hence, it is possible that there is some variation in disease clustering within neighbourhoods from one population to another and this might lead to a different optimal design in a different setting. Based on our data from Nepal, we favour the traditional EPI design with a fixed cluster size of 15, except when villages are on average further than 2 days apart, but we caution that the process of selecting an appropriate design should be done using as much local information as is available. It should also be cautioned that the EPI approach could overestimate the prevalence of xerophthalmia by up to 41% of the true prevalence, and that this is one of the disadvantages that must be weighed against the reduced survey costs attained using EPI.

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Pupillary threshold as an index of population vitamin A status among children in India[1-3]

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ABSTRACT Two hundred seven vitamin A–deficient southern Indian children aged 1–7 y (mean age: 56.9 mo) underwent testing of dark-adapted visual and pupillary thresholds in their village setting according to a previously reported protocol. One hundred thirty (62.8%) of the children also underwent serum retinol testing, and 178 (86.0%) participated in a randomized, placebo-controlled vitamin A dosing trial with pre- and postdose testing of dark-adaptation threshold. Most subjects (184 of 207, 88.9%) were able to complete pupillary testing, an objective sign requiring minimal cooperation, including a high proportion of the youngest children (72.2% of subjects aged 2 y). The proportion of children completing visual threshold testing, which requires greater understanding and cooperation, was significantly smaller than that able to complete pupillary testing (131 of 207, 63.3%; $P < 0.0001$, chi square). At baseline (predosing), the mean serum retinol concentration declined in linear fashion with a higher pupillary threshold (0.73 μmol/L with a score ≤ 4; 0.47 μmol/L with a score ≥ 8; $P < 0.01$). The mean pupillary threshold for these highly vitamin A–deficient Indian children (−0.622 log cd/m^2) was significantly higher than that for 136 more moderately deficient Indonesian children (−0.985 log cd/m^2; $P < 0.001$, two-sample t test) and 56 normal American children (−1.335 log cd/m^2; $P < 0.0001$, two-sample t test). The improvement in pupillary dark-adaptation testing was not significant for children receiving vitamin A or placebo, though there was a nonsignificant trend toward greater improvement in children receiving vitamin A ($P = 0.2$, two-sample t test). Pupillary threshold testing represents a new, noninvasive, practical, and seemingly valid approach to assessing the vitamin A status of a moderately to severely deficient preschool population. *Am J Clin Nutr* 1997;65:61–6.

KEY WORDS Vitamin A, night vision, dark adaptation, pupil, children

INTRODUCTION

Vitamin A deficiency is both the leading cause of pediatric blindness in the world today (1, 2) and a significant source of childhood mortality in the developing world (3–5). Our ability to combat vitamin A deficiency has been limited in part by the lack of a simple, practical field technique for identifying communities in need of intervention.

We recently reported encouraging results from a field trial in Indonesia of a portable, inexpensive, hand-held device designed to measure the pupillary and visual light threshold as an index of vitamin A status (6). The afferent pupillary reflex (tendency of the pupil to constrict under illumination) has been shown to correlate well with a subject's ability to detect a light stimulus near the visual threshold (7). Dark vision is known to be impaired in vitamin A deficiency, often before the appearance of other clinical signs such as Bitot's spots (8). We found that both visual and pupillary thresholds of a group of mildly vitamin A–deficient Indonesian preschool-aged children were significantly correlated with other measures of vitamin A status, including serum retinol. Most importantly, visual and pupillary thresholds rose significantly (their retinas became less light sensitive) the longer the time since a single high-dose (105 μmol) supplement of vitamin A. Thresholds subsequently fell significantly among children supplemented in randomized, double-blind fashion with vitamin A, but not among control children receiving placebo (6).

The current report presents results of pupillary and visual threshold testing on a group of children in southern India who were clinically (therefore more severely) vitamin A–deficient or the siblings of clinically deficient children, in whom deficiency is more common than in children at large (8), in contrast with the more mildly deficient, nonxerophthalmic Indonesian subjects. None of the Indian subjects had recently received vitamin A. Additionally, we tested the technique under less favorable field conditions. Although subjects in Indonesia had been tested at a single, central site, in the current study children from 40 different villages were examined.

SUBJECTS AND METHODS

Subjects and study design

A total of 213 southern Indian children aged 1–7 y ($\bar{x} \pm$ SD: 56.9 ± 15.2 mo, 55.4% male) were identified as eligible for the

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study on the basis of a history of nightblindness, the presence of Bitot's spots on clinical examination, or being the sibling of a child with nightblindness or Bitot's spots. Subjects were from a total of 40 villages in the vicinity of Madurai in the state of Tamil Nadu. We attempted both pupillary and visual testing in 207 (97.2%) of the eligible children, after informed written consent had been obtained from their parents in the local language. Blood for serum retinol analysis was obtained from 130 (62.8%) of the participating subjects (mean age: 57.1 ± 13.6 mo). Among 184 subjects (88.9%) completing pupillary testing, 178 (96.7%) participated in a placebo-controlled dosing trial in which 89 children were selected at random to receive 105 μmol vitamin A and 89 to receive placebo in double-blind fashion. All 178 subjects (100%) presented for follow-up pupillary and visual testing 2 wk after dosing (**Figure 1**). All children initially assigned to the placebo group received 105 μmol vitamin A at follow-up.

The protocols followed in testing subjects were approved by the Johns Hopkins Joint Committee on Clinical Investigation.

Apparatus and testing procedure

The hand-held illuminators used to assess pupillary and visual threshold were described previously (6). Briefly, the battery-powered machines had a yellow-green, light-emitting diode (LED) light source with 12 intensity settings at ≈0.4 log unit intervals, the range of the pupillary machine being ≈2 log units higher than the device used for visual testing. The device fit entirely over one eye, with an obliquely mounted red LED light source providing illumination of the contralateral eye. Before testing, subjects underwent a binocular partial bleach (ie, exposure to bright light to counteract previous partial dark adaptation) followed by 10 min of dark adaptation in a standard 1.5 × 2.0 × 2.0 m black-cloth tent assembled on a portable metal frame in the testing area in each village, usually a central site such as a school or clinic. The tent was set up indoors and away from windows, and its interior darkened to the degree that a dark-adapted observer was unable to read black, hand-sized letters a finger's-breadth in width on a white background. The tent was cooled internally by a battery-powered air cooler.

The visual threshold was measured first, according to a forced-choice protocol, where the threshold stimulus was defined as that level at which a subject could correctly distinguish stimulus from nonstimulus on three successive trials. The red pupillary visualization light remained off during this phase of testing to avoid confusing the subject. Next, the pupillary threshold was measured with the machine over the subject's right eye, the left eye being observed by means of a 2.5× loupe. With the subject's attention directed to a nonaccommodative target at a distance of 2 m (ie, a target at a sufficient distance to prevent accommodation or close-up focusing of the eye, which causes the pupil to constrict), the stimulus intensity was increased until a pupillary response in the contralateral eye was clearly visible to the observer on two successive trials.

All examinations were carried out in the child's village of residence by one of two workers at the Aravind Eye Hospital after 1 wk of training by one of the authors (NGC). Note that the unit of measurement used throughout this paper to denote light intensity is log cd/m^2, the metric unit for luminance, denoting the light reflected or emitted by a surface per unit area and per unit solid angle.

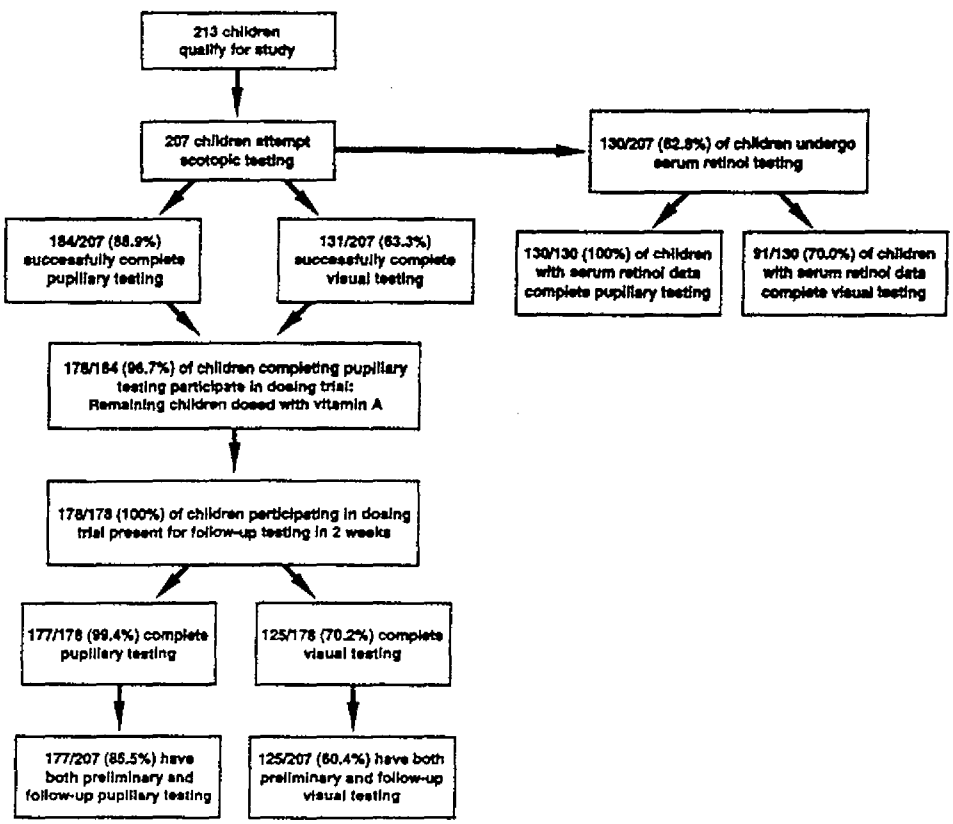

FIGURE 1. Flow chart showing the progress of 213 preschool-aged children throughout the study.

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were significantly correlated with retinol. The relation between dark-adaptation threshold tests and serum retinol concentrations was much stronger than that observed in Indonesia (6), where vitamin A deficiency was less severe, the degree of depression in dark-adaptation threshold less marked, and the range of disturbance narrower (Table 1). Nonetheless, this relation persisted even at the upper end of both the pupillary and visual curves among children who would not be expected to be vitamin A–deficient. These observations support our findings among the less-deficient Indonesian children, further suggesting that this technique for assessing dark-adaptation status may be useful in identifying populations at risk of relatively mild (subclinical) vitamin A deficiency.

In the absence of a gold standard for the assessment of vitamin A status, the randomized dosing trial provided an unequivocal means of improving vitamin A status in one group of deficient children compared with others (placebo recipients). Our previous trial in Indonesia had shown a significant improvement in the pupillary and visual dark-adaptation scores of children randomly assigned to receive vitamin A, but not of those receiving placebo. Although children randomly assigned to receive vitamin A in the current study tended to improve their pupillary scores compared with children receiving placebo, the difference was not significant. Several explanations might account for this discrepancy. We decided on a period of 2 wk for retesting postdosing on the basis of animal (10) and human (11, 12) studies that suggested that this would be adequate for return to baseline dark adaptation, a period that appeared to have been sufficient in Indonesia. In view of the more severe degree of vitamin A deficiency among the Indian subjects, a longer postdosing interval may have been needed. Whereas some sources indicate a shorter time for resolution of nightblindness after dosing in less-severe cases of deficiency (8), other studies have suggested that multiple doses of vitamin A may be needed to reverse the effects of xerophthalmia in highly deficient subjects (13, 14). Finally, deficiencies of other nutrients known to be involved in vitamin A release and utilization (eg, zinc) might have been present. In view of the potential effect of vitamin A supplementation on mortality in this age group, it was not felt ethical to withhold dosing from children assigned to the placebo group for > 2 wk. Longer follow-up of both groups might have been revealing, however.

The ability to identify populations in need of vitamin A intervention requires a simple, inexpensive, and practical field technique for assessing vitamin A status in preschool children (the group most likely to be severely deficient). Pupillary dark-adaptation testing provides distinctly different results in populations with presumably normal (eg, United States), moderately deficient (Indonesia), and severely deficient (southern India) vitamin A nutriture. This mode of testing offers obvious advantages over currently available techniques for the assessment of a population's vitamin A status, given that results are immediate, it is noninvasive, and that blood samples do not require delicate transport, storage, and sophisticated analysis. Future testing might be made even more efficient by eliminating the flash, which calculations have shown to bleach < 10% of available rhodopsin, and by allowing children in groups of a dozen or so to dark-adapt simultaneously and maximally (eg, for 30 min). In this way, the time for full evaluation of each child would be reduced to the ≤ 5 min required for the testing itself, without separate, sequential dark-adaptation periods for each subject.

The smaller the sample size required to determine whether a stated level of vitamin A deficiency exists, the more practical the test. Among the various factors that determine sample size, the prevalence of the condition being assessed and the validity of the test itself (the level of "background noise") are both important. Detection of impaired dark adaptation should, theoretically, have advantages in this regard. It represents a demonstrable physiologic consequence of deficiency, and, because it occurs at relatively mild levels of deficiency, is more prevalent than more severe clinical manifestations. For example, examination of the mean (± SD) dark-adaptation thresholds for Indian, Indonesian, and American children allowed us to calculate the minimum number of children required to show a difference between populations at, eg, the 0.01 level of significance. A sample of 27 Indonesian children would have been adequate to show a difference between the Indonesian and American populations, and only 7 Indian children would be needed to show a difference between the American population and the much more deficient Indian population. In comparison, identifying deficiency with the use of more severe clinical indicators requires the examination of hundreds to thousands of children (15). In interpreting these numbers, it is necessary to remember that the populations tested in both Indonesia and India are not representative of average preschool-aged children in those countries, but were rather chosen to be deficient in vitamin A. Among more borderline populations, larger numbers of subjects would presumably need to be tested to establish deficiency.

Obviously, testing a larger and more representative group is necessary to determine the extent and severity of deficiency and to provide an adequate baseline for assessing the impact of interventions. Only by testing additional populations of varying vitamin A status will it be possible to determine appropriate mean dark-adaptation thresholds to serve as valid criteria for differing degrees of deficiency, and as the basis for determining when an intervention program is warranted and whether or not it is having its intended effect.

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Effect of vitamin A supplementation on measles vaccination in nine-month-old infants

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Objective: Childhood immunization programs have been suggested as an infrastructure to deliver vitamin A supplements to children in developing countries. The effects of giving vitamin A, a potent immune enhancer, with measles immunization to nine-month-old infants is unknown.
Methods: A randomized, double-masked, placebo-controlled clinical trial of vitamin A, 100 000 IU at the time of standard titer Schwarz measles immunization was conducted with nine-month-old infants in Bogor District, West Java, Indonesia. Antibody titers to measles were measured at baseline and one and six months following immunization.
Results: 394 infants received measles immunization, and 37 infants (9.4%) had baseline antibody titers > 1:120, which is consistent with previous natural measles infection. Of the remaining infants, 98.8% seroconverted to measles, and 99.3% had titers consistent with protection against measles six months postimmunization. Seroconversion rates were similar in vitamin A and placebo treatment groups.
Conclusion: High dose vitamin A supplementation can be given without reducing seroconversion to standard titer Schwarz measles immunization in nine-month-old infants.

Keywords: vitamin A; retinol; measles; vaccination; Indonesia

Introduction

Vitamin A deficiency is a major cause of childhood morbidity and mortality in many developing countries worldwide. Periodic vitamin A supplementation has been shown to reduce child mortality by 20–50%[1] and is one of the most cost-effective interventions available to increase child survival.[2] Child immunization programs involve more than 500 million contacts per year, and it has been suggested that the infrastructure of these programs could be used to deliver vitamin A supplements.[3] Vitamin A is a potent immune enhancer.[4] In a recent study, vitamin A supplementation with measles vaccination was shown to reduce seroconversion to measles in six-month-old infants who had high levels of passively acquired maternal antibody to measles.[5] Many child immunization programs in developing countries routinely immunize against measles at nine months of age, a time at which the levels of maternal antibody are generally lower. The effect of vitamin A supplementation on measles vaccination in nine-month-old infants is not known. We conducted a randomised, double-blind, placebo-controlled clinical trial to determine the effect of simultaneous vitamin A administration on antibody responses to measles vaccination in nine-month-old infants.

Methods

The study population consisted of infants in 27 villages in the Bogor District, West Java, Indonesia. This area has a high prevalence of subclinical vitamin A deficiency.[5] Births were monitored in the area with the assistance of the rural health centers. The overall design of the study was to evaluate the impact of integrating vitamin A supplementation with child immunization programs, starting with the early immunization contacts. Infants were enrolled into the clinical trial between October, 1992, and February, 1993, and were randomly allocated by number table in blocks of ten to receive either 25 000 IU or 50 000 IU, vitamin A, at 6, 10, and 14 weeks immunization contacts of the Expanded Programme on Immunization and vitamin A, 100 000 IU at nine months of age, or placebo at 6, 10, and 14 weeks, and nine months of age. Standard-titer Schwarz measles vaccine, 0.5 mL (Morbilivax, lot 68A09, 4.13 log10 median tissue-culture infectious doses [TCID50] per dose, Biocine Sclavo, Siena, Italy), was administered at nine months of age by three pediatricians. Vitamin A, 100 000 IU, or placebo in identical capsules (Task Force Sight and Life, Basel, Switzerland) was given orally at the same time as measles immunization. Infants received identification numbers as they were enrolled into the study, and each identification number had an envelope with an identical capsule containing either vitamin A or placebo. At the time of treatment allocation, both pediatrician and study nurse were required to verify the identification number of the infant. Weight, height, and mid-upper-arm circumference were measured by an anthropometrist at 6 and 14 weeks, and 10 and 15 months of age. All infants received a booster immunization six months after the first measles immunization.

Blood was obtained by venipuncture at all three visits, and plasma was separated and frozen at −70°C. Antibody titers to measles were measured by plaque reduction neutralisation assay at the Division of Viral Products, US Food and Drug Administration, Bethesda, Maryland, and were standardized against measles reference serum (World Health Organization, Geneva). Seroconversion to measles was defined as four-fold or greater rise in antibody titer

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over the calculated expected titer.[6] The half-life of passively acquired maternal antibody was considered to be four weeks. Baseline antibody titers of > 1:120 at nine months were considered to indicate previous natural measles exposure.[6] Titers < 1:8 were regarded as 1:4 for calculation of seroconversion rates and geometric mean titers. The antibody level considered to be consistent with protection six months after immunization was defined as a titer ≥ 1:120.[7] Categorical analyses were conducted using chi-square and exact tests. Student's and Bonferroni's *t* tests were used for appropriate comparisons of continuous data. All procedures were approved by the appropriate ethical committees in Indonesia and the United States.

Results

The study enrolled 467 infants at 6 weeks of age, and 394 infants (84.7%) were present at the time of measles immunization at 9 months of age. Of the infants who were immunized, the follow-up rates were 95.2% and 78.7% at one and six months after immunization, respectively. As shown in Table 1, there were no significant differences between the three treatment groups in age, sex, weight, height, and mid-upper arm circumference.

Thirty-seven of 394 infants (9.4%) had preimmunization titers at nine months of age > 1:120, consistent with a previous history of natural measles infection,[6] and this group was analyzed separately from infants with baseline titers ≤ 1:120. Of the remaining 343 infants who were followed to one month postimmunization, 98.8% seroconverted to measles. Of 285 infants followed six months postimmunization, 99.3% had titers consistent with protection against measles. Similar rates of seroconversion to measles at one month postimmunization and protection to measles at six months postimmunization were observed in all three treatment groups (Table 2)

Geometric mean titers before and after measles immunization in the three allocation groups are shown in Figure 1. There were no significant differences in geometric mean titers to measles at one and six months postimmunization in the three allocation groups. Of the 212 boys and 182 girls who received measles immunization, the geometric mean titers were 1:1920 and 1:1571 ($P = 0.07$) at one month postimmunization, and 1:1164 and 1:1053 ($P = 0.29$) at six months postimmunization, respectively. Fifty of the 357 infants (14%) had preimmunization titers at nine months of age > 1:8 and < 1:120. Geometric mean titers were 1:1772 and 1:2298 ($P = 0.18$) at one month postimmunization, and 1:1164 and 1:1900 ($P = 0.03$) at six months postimmunization in the infants receiving vitamin and placebo, respectively.

Discussion

This study demonstrates that vitamin A supplementation does not reduce seroconversion to standard titer Schwarz

Table 1 Characteristics of infants in the treatment groups

Characteristic	Vitamin A ($n = 132$)	Vitamin A ($n = 132$)	Placebo ($n = 130$)
Dose of vitamin A at 6, 10, 14 weeks	50 000 IU	25 000 IU	placebo
Dose of vitamin A at 9 months	100 000 IU	100 000 IU	placebo
Age (D)[a]	279 (12)	278 (14)	280 (11)
Sex	72 M, 60 F	74 M, 58 F	66 M, 64 F
Weight (kgs)[a]			
14 weeks	6.1 (0.8)	6.1 (0.9)	6.1 (0.8)
10 months	8.0 (1.0)	8.1 (1.0)	8.1 (0.9)
Height (cms)[a]			
14 weeks	60.3 (2.5)	60.4 (2.5)	60.3 (2.1)
10 weeks	69.1 (2.5)	69.2 (2.6)	69.2 (2.3)
Mid-upper arm circumference (cms)[a]			
14 weeks	13.5 (1.2)	13.5 (1.3)	13.7 (1.3)
10 months	14.3 (1.2)	14.5 (1.3)	14.4 (1.1)

[a]Mean ± s.d.

Table 2 Seroconversion and protection against measles after immunization[a]

	Vitamin A supplementation prior to and at time of vaccine[a]		
Response to measles immunization:	Placebo × 3 Placebo	25 000 IU × 3 100 000 IU	50 000 IU × 3 100 000 IU
Prevaccination titer < 1:8			
Infants seroconverted	95 of 96 (99%)	104 of 106 (98%)	90 of 91 (99%)
Infants protected	78 of 79 (99%)	90 of 91 (99%)	79 of 79 (100%)
Prevaccination titer 1:8 to 1:120			
Infants seroconverted	21 of 21 (100%)	10 of 10 (100%)	19 of 19 (100%)
Infants protected	14 of 14 (100%)	8 of 8 (100%)	14 of 14 (100%)
Prevaccination titer > 1:120			
Infants seroconverted	1 of 11 (9%)	1 of 8 (12%)	1 of 13 (8%)
Infants protected	11 of 11 (100%)	8 of 8 (100%)	6 of 6 (100%)

[a]Seroconversion defined as a four-fold or greater increase in titer over the expected titer at one month post-immunization. Protection defined as a serum neutralizing antibody titer > 1:120 at six months post-immunization.

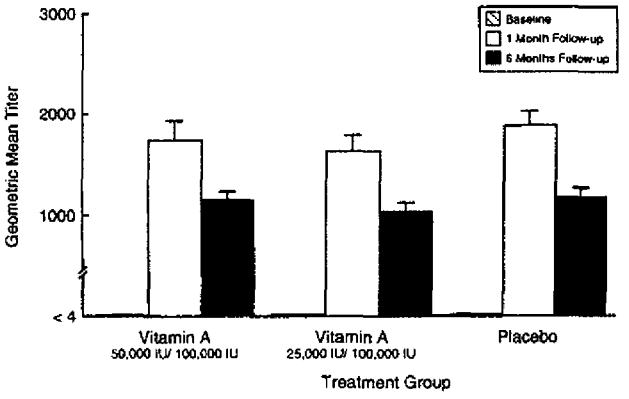

Figure 1 Geometric means titers to measles at baseline and one and six months post immunization by allocation group. The vertical bars represent standard error of the mean. No significant differences, as assessed by Bonferroni's *t*-test, were noted between treatment groups at one and six months postimmunization.

measles immunization in nine-month-old infants in this study population in Indonesia. Our previous study showed that vitamin A supplementation was more likely to interfere with seroconversion if passively acquired maternal antibody to measles was present at the time of measles immunization. Only 14% of infants had maternal titers to measles at the time of immunization at nine months of age. Among the infants who had measles antibody prior to immunization with titers in the $> 1{:}8$ to $\leq 1{:}120$ range, there seemed to be a reduction in geometric mean titers to measles at one and six months postimmunization, which corroborates our previous findings. However, all infants with titers in the $> 1{:}8$ to $\leq 1{:}120$ range seroconverted one month following immunization, regardless of whether they received vitamin A or placebo. Infants who had preimmunization titers of $> 1{:}120$ in general did not show a fourfold or greater increase in titer one month following immunization, and this antibody response is consistent with infants who had a previous natural measles infection.

At nine months of age, 9.4% of the infants had preimmunization titers of $> 1{:}120$ which suggested a previous natural measles infection. In the same community in Indonesia, only 1.5% of six-month-old infants had preimmunization titers consistent with previous measles infection.[5] Although about 99% of nine-month-old infants without previous natural measles infection seroconverted to measles immunization, these data suggest that the timing of measles immunization may need further evaluation. If one assumes that approximately 10% of infants will have natural measles infection prior to measles immunization at nine months of age and a measles case fatality rate of 20–30%, there could potentially be 20–30 deaths per 1000 infants attributable to measles infection prior to immunization.

The levels of passively acquired maternal antibodies to measles in infants is likely to depend on complex patterns of natural measles infection and measles vaccination within communities. It is unclear whether these findings can be extrapolated to populations in areas such as sub-Saharan Africa and Central and South America, and further studies are in progress to verify these findings in other populations. This study suggests that the Expanded Programme on Immunization could include vitamin A supplementation with measles immunization at nine months of age without interference with seroconversion. The benefits of vitamin A supplementation in reducing child morbidity, mortality, and blindness in developing countries have been well described.[8]

Acknowledgements

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Statistical methods

SAS programs (SAS, Inc, Cary, NC) were used to measure the association between pupillary and visual threshold and other indicators of vitamin A status, and between the change in postdosing threshold and a subject's assignment in the placebo-controlled dosing trial. The two-tailed Student's t test was used to compare means between two groups, whereas the chi-square test was used to compare proportions. The chi-square test of trend was used to assess the significance of association between the two dark-adaptation tests and serum retinol. Analysis of variance (ANOVA) was calculated for a model comparing the population mean dark-adapted thresholds for American, Indonesian, and Indian children. The minimum number of Indian and Indonesian subjects that would have to be tested to establish a significant difference in mean threshold at the 0.05 level between these groups and normal American children with a power of 99% was calculated with a standard sample-size formula. A two-tailed probability < 0.05 was considered significant.

RESULTS

Subject compliance

Most subjects (184 of 207, 88.9%) were able to complete pupillary testing, including a high proportion of the youngest children (13 of 18, 72.2% of subjects aged 2 y). The proportion of children completing pupillary testing was higher than that for visual testing at all ages, with the overall difference in compliance between the two tests being highly significant ($P <$ 0.0001, chi-square; **Figure 2**). The difference in level of compliance between the two tests was most marked among the youngest children, who generally complied poorly with visual testing (Figure 2).

Correlation between threshold testing and vitamin A status

Mean serum retinol declined linearly with increasing pupillary threshold scores (lower retinal sensitivity) (**Figure 3**; $P <$ 0.01, chi-square test of trend). A similar monotonic trend was observed for mean serum retinol and visual threshold, although this relation was not significant (Figure 3; $P < 0.3$, chi-square test of trend). Age, sex, breast-feeding status, and a recent (previous 2 mo) episode of mumps, measles, cough, or diarrhea were not significantly correlated with serum retinol or dark-adaptation score on linear modeling (data not shown).

Children reported by mothers to be nightblind did not have significantly lower serum retinol concentrations (0.57 ± 0.27 compared with 0.61 ± 0.25 μmol/L; $P = 0.4$, Student's t test) or higher (less sensitive) pupillary threshold scores (−0.55 compared with −0.67 log cd/m^2; $P = 0.25$, Student's t test), although the relations were in the expected direction. All children either had evidence suggesting clinical vitamin A deficiency (a history of nightblindness or current Bitot's spots) or were the siblings of deficient children, who are themselves more likely to be deficient (8).

Results of a randomized, placebo-controlled dosing trial

For 89 children receiving high-dose vitamin A supplements, pupillary dark-adaptation scores improved by an average of

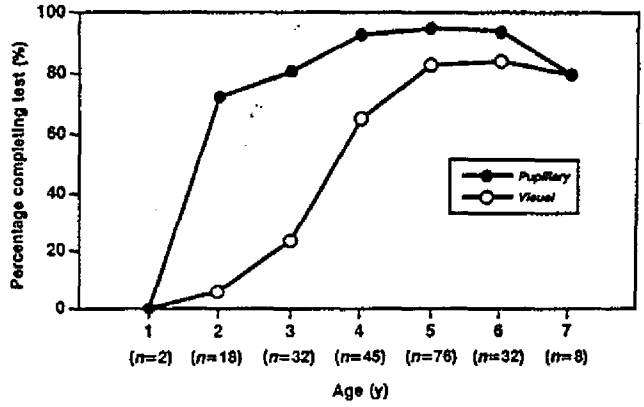

FIGURE 2. Compliance with pupillary and visual testing by age (n = number of subjects attempting testing). The difference between the two curves was significant, ($P < 0.0001$, chi-square test).

0.159 ± 0.60 log cd/m^2, whereas for children receiving placebo, scores improved by only 0.016 ± 0.50 log cd/m^2. Neither the change in score for children receiving vitamin A ($P = 0.15$, paired t test), nor that for subjects receiving placebo ($P = 0.7$, paired t test), nor the difference in improvement between the two groups ($P = 0.20$, two sample t test) was significant. There was no significant difference in the change in visual scores between vitamin A and placebo recipients (0.020 ± 0.59 and −0.013 ± 0.54 log cd/m^2 respectively; $P = 0.50$, two-sample t test) (**Figure 4**). Resistance to multiple blood draws among children and their parents made postdose serum testing impractical.

Comparisons between different populations

The children tested in India were more vitamin A–deficient than the children of similar age tested in Indonesia: the mean serum retinol concentration was significantly lower ($P <$ 0.0001, two-sample t test), and the proportion of children with a serum retinol concentration < 0.70 μmol/L ($P < 0.0001$, chi-square test) and reported to be nightblind ($P < 0.0001$, chi-square test) were significantly higher for the Indian children (**Table 1**).

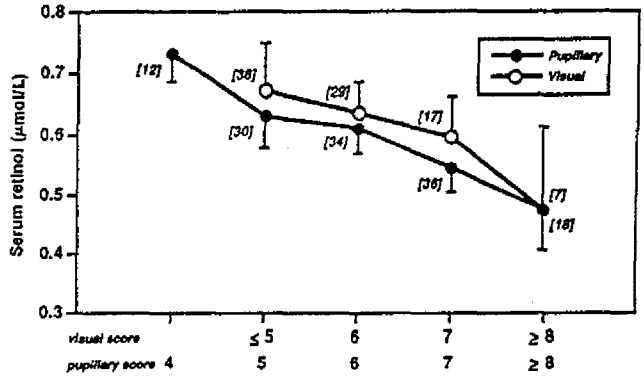

FIGURE 3. Mean (± SE) serum retinol concentrations by pupillary and visual dark-adaptation scores (number of subjects in brackets). The apparent decline in retinol with higher (less sensitive) scores was significant for pupillary score ($P = 0.01$, chi-square test of trend) but not for visual score ($P = 0.21$, chi-square test of trend).

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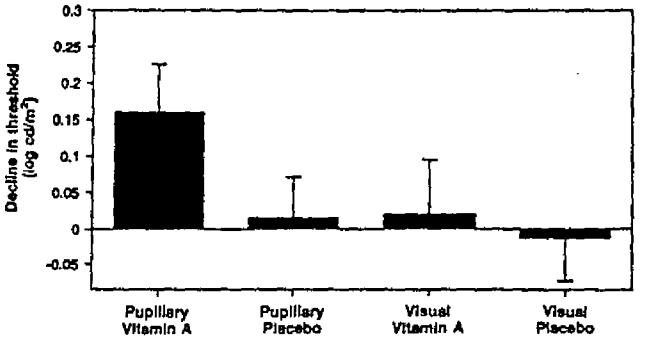

FIGURE 4. Mean (± SE) decline in dark-adaptation thresholds after supplementation with vitamin A or placebo. The difference in decline was not significant between individuals receiving placebo or vitamin A for either the pupillary ($P = 0.2$, t test) or visual ($P = 0.5$, t test) tests.

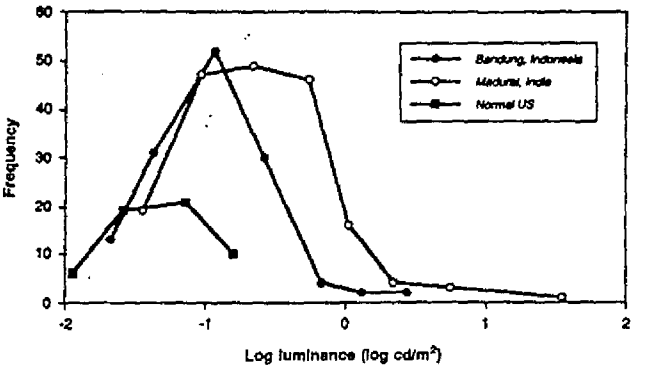

FIGURE 5. Pupillary scores for severely vitamin A–deficient Indian children compared with scores for moderately deficient Indonesian and normal American children. The mean score for Indian children (−0.622 log cd/m²) was significantly higher (poorer dark adaptation) than the score for Indonesian children (−0.985 log cd/m²; $P < 0.001$, t test) and normal US children (−1.335 log cd/m²; $P < 0.0001$, t test).

As anticipated, the apparently poorer vitamin A status of the Indian children was associated with a higher mean pupillary threshold (lower retinal sensitivity) compared with the Indonesian children (−0.622 compared with −0.985 log cd/m²; $P < 0.001$, two sample t test), who in turn showed significantly poorer dark adaptation than a group of 56 apparently well-nourished American children aged 54 ± 18 mo with dark irides (two-sample t test: −1.335 log cd/m², $P < 0.0001$; comparison of the three populations by ANOVA: $F = 55.95$, $P < 0.0001$) (**Figure 5**). Although serum retinol was not measured in the American subjects, the extremely low prevalence of vitamin A deficiency among well-nourished American children is well-documented (9). American children with dark irides were chosen because we found previously (unpublished data) that American children with light irides showed a slightly more sensitive response to pupillary threshold testing.

Although pupillary data differed significantly between the Indian and Indonesian populations, visual threshold data did not (−4.00 compared with −4.09 log cd/m²; $P = 0.5$, two sample t test). Comparable visual threshold data were not recorded for the American subjects.

DISCUSSION

This study is a sequel to our previous clinic-based assessment of this new test of dark adaptation, with measurements made under actual village-based conditions. All testing was conducted in a portable testing site, constructed of locally available materials and reassembled in each of the 40 villages, which were within a 3-h drive of our base at the Aravind Eye Hospital. The inability of a dark-adapted observer to read standard-sized test letters inside the test site guaranteed a reproducible, minimum level of darkness. Our results indicate that this technique is sufficiently robust to allow valid testing under these conditions.

As had been the case in Indonesia (6), a very high proportion of children completed pupillary dark-adaptation testing, significantly greater than the percentage able to comply with visual testing. Pupillary testing, in which the response was objectively graded by an observer, was more useful among young children, especially children 4 y of age and younger, the group at greatest risk for vitamin A deficiency.

Our data suggest that pupillary testing is not only more acceptable to young children, but may also provide a more accurate indication of impaired dark adaptation: pupillary threshold was significantly correlated with serum retinol whereas visual threshold was not. If pupillary testing does indeed prove to be a more accurate indicator of vitamin A status in this age group, it may well be due to practical issues of testing rather than to any fundamental difference between the two tests. Both pupillary and visual testing assess the function of the dark-adapted retina, known to be impaired in vitamin A deficiency. It is possible that further experience will show that the two tests provide complementary information, but we are unaware of any data to suggest that either offers a theoretical advantage in the assessment of vitamin A status.

The degree of success in pupillary threshold testing is notable, given that children in this part of India are quite unused to sitting in darkened conditions, and that testing was carried out during the hottest part of the year. These problems were in part overcome by designing a portable testing area that was large enough to accommodate the subjects' mothers, and that could be made comfortable by means of an electric air cooler weighing < 10 kg and 70 cm in height.

As expected, mean serum retinol declined in linear fashion with higher (less sensitive) scores on both pupillary and visual dark-adaptation testing, although only the pupillary scores

TABLE 1
Vitamin A status of 130 Indian children compared with 136 Indonesian children

Indicator	Madurai, India	Bandung, Indonesia[1]
Age (mo)	56.9 ± 15.2[2]	58.5 ± 7.0
Serum retinol (μmol/L)	0.59 ± 0.26	0.85 ± 0.34[3]
Proportion with retinol < 0.7 μmol/L (%)	92/130 (70.8)	47/122 (38.5)[4]
Proportion reported as nightblind (%)	93/207 (44.9)	0/136 (0)[4]

[1] Six months after receiving a baseline vitamin A dose of 105 μmol.
[2] $\bar{x}$ ± SD.
[3,4] Significantly different from Madurai, India: [3] $P < 0.0001$ (Student's t test), [4] $P < 0.0001$ (chi-square test).

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Vitamin A prophylaxis

It has been little more than a decade since the initial observation of the dose dependent relation between the severity of vitamin A deficiency and childhood mortality,[1] quickly followed by the publication of a controlled trial in which children of preschool age, randomised to receive large doses of vitamin A every six months, died at only two thirds (or less) the rate of control subjects.[2 3] In the short interval since this trial, an initially sceptical scientific community has declared control of vitamin A deficiency a major international goal[4-6] and potentially one of the most cost effective of all health interventions.[7]

The story did not begin in the 1980s. A host of animal studies and anecdotal clinical reports during the first third of the century, soon after vitamin A was discovered, suggested a close, potentially causal relation between vitamin A status and morbidity and mortality from infection. These are detailed elsewhere.[8]

Vitamin A prophylaxis and mortality

For ethical and logistic reasons the observational study[1] has never been repeated, though a large number of intervention trials have been carried out. Eight were initially considered to be suitably rigorous for inclusion in an independently commissioned meta-analysis (table 1).[9] The results were remarkably similar, particularly given the wide differences in culture, dietary habits, disease patterns, and malnutrition of the populations studied, the differences in study design, and the variation in the potential effectiveness of the strategies used to improve vitamin A status.[2 10-16]

On an intent to treat basis, six of the eight studies recorded a statistically significant reduction in mortality among children assigned to receive vitamin A supplements (19 to 54%), even though not all those assigned vitamin A complied with the treatment.[2 8]

Negative results

Two 'outlier studies', in which no reduction in mortality was observed, deserve mention. The Hyderabad trial[13] had a series of problems that only became apparent after publication from an exchange of letters to the editor: children were routinely examined and treated for disease each week by specially trained health workers. This may explain why both the treatment and control arms experienced mortality much lower than anticipated. This general reduction in mortality drastically reduced the power of the study to detect an effect attributable to the vitamin A supplement (mean reduction 6%, 95% confidence interval

−50 to 50%).[9] In addition, the study had a large and differential loss to follow up and low levels of compliance.

The Sudan trial[16] probably did not establish a meaningful difference in vitamin A status between its two study groups. It is likely that the study children were not particularly deficient to begin with: almost half lived in homes with sanitary facilities and piped-in water, hardly representative of the economic status and living conditions of most children in the developing world.

Importantly, the senior authors of both these studies have subsequently supported the value of improving vitamin A status as a means of reducing childhood mortality.[8 17]

Infectious morbidity and mortality

Longitudinal observational data have suggested that vitamin A status can affect the incidence of infectious diseases.[18 19] Intervention trials, however, have not confirmed a causal relation; instead, improving vitamin A status primarily reduces the severity of infectious episodes,[14 20] particularly life threatening diarrhoea and measles (table 2).

Measles

In the four community based prophylaxis trials in which cause specific mortality was ascertained, mortality from measles was reduced by roughly 50% (table 2).[11 12 14 15] In the one trial which did not arrive at this conclusion,[15] the data presented in the published paper clearly prove otherwise (though given the sample size the large clinical difference was not statistically significant). Remarkably, the treatment with high doses of vitamin A of children admitted to hospital with moderate to severe measles reduces mortality to a similar extent,[21-23] strongly suggesting that the beneficial impact is secondary to the correction of the underlying vitamin A deficiency and not to a non-specific adjuvant response related to the large dose.[8] Treatment with large doses of vitamin A (200 000 IU on two successive days) also reduces the severity and persistence of complications related to measles.[22-25]

Diarrhoea

Children assigned to the vitamin A supplementation arms of the community based mortality trials had one third fewer deaths attributable to diarrhoea than the control subjects (table 2). Community based studies on the effect of supplementation on morbidity support these results: although supplementation did not reduce the incidence of diarrhoea, it did reduce the severity of subsequent diarrhoeal episodes.[4 20] The lack of apparent impact on incidence may be real; alternatively, it may reflect an inadequate sample size given the high frequency of trivial diarrhoeal episodes among children in the developing world.[8]

Respiratory disease

Despite evidence for an association between vitamin A deficiency and the prevalence and incidence of respiratory disease,[8 18] the prophylaxis trials did not show a consistent impact on death from respiratory disease (table 2). Indeed, it has been suggested that vitamin A supplementation may increase the risk of respiratory infection, though a World Health Organisation Consultative Group, after reviewing all available data, concluded that this was unlikely.[26] Some workers have suggested that the apparent contradiction may reflect the reversal of squamous metaplasia of the epi-

Table 1 Major community mortality prevention trials

Study	*Country*	*Vitamin A supplement*	*Reported mortality reduction (%)**	*Primary reference*
Aceh	Indonesia	Large dose every six months	34†	Sommer *et al*[8]
Bogor	Indonesia	Vitamin A fortified monosodium glutamate	45	Muhilal *et al*[10]
NNIPS	Nepal	Large dose every four months	30	West *et al*[11]
Jumla	Nepal	One large dose, follow up at five months	29	Daulaire *et al*[12]
Tamil Nadu	India	Weekly moderate dose	54	Rahmathullah *et al*[15]
Hyderabad	India	Large dose every six months	6 (not SS)‡	Vijayaraghavan *et al*[13]
Khartoum	Sudan	Large dose every dix months	(+6; not SS)	Herrera *et al*[16]
VAST	Ghana	Large dose every four months	19	Ghana VAST Study Team[14]

* 6 months and older at baseline (1 year or older if younger children not reported separately).
† Alternative analyses suggest at least 40 to > 50%.
‡ As calculated from data in their publication, but not reported as such.[9]
SS = statistically significant ($p < 0.01$).
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Table 2 Cause specific mortality, vitamin A supplementation community prevention trials

	Symptoms/diseases					
	Measles		*Diarrheoa*		*Respiratory*	
Study	*Vitamin A*	*Control group*	*Vitamin A*	*Control group*	*Vitamin A*	*Control group*
Tamil Nadu[15]						
No of deaths	7	12	16	33	2	3
RR*	0.58		0.48		0.67	
NNIPS[11]						
No of deaths	3	12	39	62	36	27
RR	0.24		0.61		1.29/1.00†	
Jumla¶[12]						
No of deaths	3	4	94	129	18	17
RR	0.67		0.65		0.95‡	
Ghana[14]						
No of deaths	61	72	69	111	47	45
RR	0.82		0.66§		1.00	

* RR (relative risk): cause specific mortality of vitamin A group divided by mortality in control group.
† Original published results[11]: RR=1.29; reanalysis as an associated cause that recognises underlying causes; RR=1.00 (KP West, unpublished data).
‡ Pneumonia case management programme may have confounded results.
§ Defined as 'acute gastroenteritis'.
¶ Except for Jumla, findings relate to children already ≥ 6 months of age when supplemented.
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thelial lining of the respiratory tract, increasing its ability to mount a clinically detectable response to an infection. A definitive answer awaits further investigation.

Young infants

Few data suggest that vitamin A supplementation dramatically reduces childhood mortality in infants younger than 6 months of age. The only carefully conducted community based prophylactic trial even suggests the potential for a small increase in mortality among children supplemented with vitamin A during the second and third months of life; clear cut protection is evident by the age of 5 months.[8 27] Whether this is a real effect or chance variation awaits the results of follow up investigations.

In one trial Indonesian children randomised to receive 50 000 IU vitamin A at birth had only half the first year mortality of control subjects.[28] Almost all the benefit occurred during the second to fourth months of life.

Non-infectious morbidity

Apart from infectious disease, vitamin A status appears to play an important part in growth and haemoglobin synthesis. Human data on growth, particularly from controlled intervention trials, are confusing: from no impact at all to an age dependent influence on ponderal and/or linear growth.[8] Presumably vitamin A can be a limiting factor of many aspects of the growth process, but the degree (if any) depends on the relative adequacy of other nutrients. In contrast, numerous studies have shown that improvement in vitamin A status favourably affects iron metabolism, reducing the severity of anaemia.[8 10 28]

Mechanism of action

The protective effects of vitamin A supplementation seem to be entirely related to restoring normal vitamin A status. This, in turn, increases resistance to (severe) infection through at least two mechanisms: the restoration of normally differentiated epithelia, providing a more effective barrier to infection; and up-regulation of immune competence.[8]

For example, children admitted to hospital with severe measles who were randomised to vitamin A supplementation developed a far greater immune response than control subjects.[25] The speed of the protective response, graphically illustrated by the 50% reduction in mortality from measles among children supplemented with vitamin A after admission to hospital, seems at first glance surprising. We now know, however, that vitamin A regulates the expression of at least 300 different genes and that the nasogastric administration of vitamin A to deficient rats results in detectable alterations in gene products within an hour.[8] Hence the dramatic clinical response observed in hospital and field studies has a readily demonstrable biological basis, even if it is only currently partially understood.

Recommendations for prophylaxis

The ultimate goal of prophylaxis is to restore normal vitamin A status to deficient children. The most effective means for accomplishing this urgent task will depend on the vagaries of local culture, the available foods, and the local health system. Ideally, every child (and woman) should receive adequate vitamin A as part of their regular diet. Small daily doses are more efficiently absorbed and stored than large periodic supplements. Unfortunately, the population of those countries in which the problem is most severe and extensive subsist primarily on vegetable diets, containing little, if any, preformed vitamin A (aside from breast milk, which is an excellent source for the young infant). Provitamin A carotenoids found in many fruits and vegetables are inefficiently converted to the active agent and one of the best, most widely available sources of provitamin A carotenoids, dark green leafy vegetables, is commonly eaten in only small amounts, if at all, by young children.

Key messages

- Vitamin A deficiency increases the severity of and mortality from measles and diarrhoea
- Increased infectious morbidity and mortality is apparent even before the appearance of xerophthalmia
- Improving the vitamin A status of deficient children aged 6 months to 6 years can dramatically reduce their morbidity and mortality from infection
- Prompt administration of large doses of vitamin A to children with moderate to severe measles, particularly if they may be vitamin A deficient, can reduce individual mortality by 50% and prevent or moderate the severity of complications

Many wealthier countries rid themselves of the problem through the fortification of dietary staples, particularly margarine and bread. Fortification of sugar has proved to be an effective approach for dramatically reducing the prevalence and severity of vitamin A deficiency in Guatemala and is now being instituted in a number of other Latin American countries.

Where the problem is most severe, however, particularly in Africa and Asia (India, Bangladesh, Pakistan, Indonesia, and the Philippines), periodic supplementation of every child once every three to six months (100 000 IU at less than 1 year of age; 200 000 IU for older children) is recommended. Children at special risk (for example severe protein energy malnutrition, chronic diarrhoea, repeated respiratory infection) are provided with an additional dose if they have not received routine prophylaxis within the past month. Measles is treated with a 200 000 IU supplement on two successive days as a medical emergency. To boost maternal stores and the amount of vitamin A in breast milk, women are advised to receive 200 000 IU within four to six weeks of delivery.

Over 60 countries are now planning, or have instituted, programmes to control vitamin A deficiency. Periodic supplementation as a special endeavour generally achieves sustainable coverage rates of 40–60%, though some countries (and most demonstration projects) attain far higher levels. In Indonesia, where distribution has been integrated into a burgeoning health service system and mass media has been used to educate the public and change dietary patterns, a 90% reduction in the prevalence of overt deficiency has been achieved.[8]

Unquestionably, the major challenge remains the design and implementation of effective population based intervention programmes.

Conclusions

Although there is a great deal more to be learnt about the value of vitamin A status and childhood morbidity and mortality, there is no longer any credible doubt that deficiency is inimical to optimum health and survival. These effects begin to occur even before the appearance of ocular disease ('xerophthalmia').[8 29]

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The Duration of the Effect of Vitamin A Supplementation

The recent article by Ross et al.[1] further analyzes data from the carefully conducted vitamin A intervention trials to garner *evidence* for whether large dose supplementation at 4-month intervals provides optimal protection against excess morbidity and mortality associated with vitamin A deficiency. Finding no significant decrement in impact in relation to either the duration between last dose or cumulative number of doses and subsequent mortality, the authors, and an accompanying editorial,[2] suggest the 4-month interval may be optimally effective.

On the face of it, the results are indeed reassuring. However, they raise another question. If a 4-month interval is maximally effective, and if the impact of supplements occurs by improving vitamin A status that is initially suboptimal, why does repeated periodic dosing work at all? Either there really is room for further improvement in vitamin A status and therefore its impact on morbidity and mortality (which seems most likely, since a host of biochemical studies clearly demonstrate the transient impact of periodic supplements on biochemical indices of improved status),[3] or the large dose is exerting its impact through a bolus effect by "spiking" vitamin A status. The latter seems equally unlikely given the brief period of "normal status" enjoyed by supplemented children versus the prolonged benefit that ensues.

These considerations suggest that just as in prevention of xerophthalmia,[4] a periodic large dose is protective longer than measures of "biochemical indices" would suggest.[5] Hence the 4-month interval provides a sustained rate of protection, but probably one that is not as great as it would be if the child were truly vitamin A replete.

The editorial[2] misses this important point and compounds the problem by suggesting that the 4-month interval is highly protective, while a 6-month interval probably is not. The evidence presented to support this conclusion rests on the absence of observed protection in two trials using a 6-month interval in comparison with one in which a 34% (to 70%)[6,7] protection was observed. A number of alternative explanations have been offered to explain the absence of an observable impact (at any interval following supplementation) in the two aforementioned studies.[3,8] The simplest reasoning, however, belies the editorialist's explanation: if 4 months is accepted as an effective interval, then even if 6 months were less optimal, one would expect two-thirds the benefit of the 4-month interval rather than no benefit at all! Expressed differently, the expected benefit during months 1 through 4 would need to be cancelled by a negative impact, of greater magnitude, during months 5 and 6.

In conclusion, it is reassuring to learn that in the Ghana studies no diminution occurred in protection from excess morbidity and mortality with increasing duration from dosing. Four-month (or shorter) intervals between doses (the optimal being daily, smaller doses) over 6 months seems self-evident. While this analysis does not demonstrate that, it does raise the question why the vitamin A recipients exhibited any benefit from sequential dosing if 4-month intervals were optimal. □

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Vitamin A Deficiency, Child Health, and Survival

While populations of wealthier countries seek the putative benefits of near-pharmacologic levels of antioxidants (including vitamin A) as protection from chronic diseases associated with aging, scientific evidence accumulated over the past two decades has confirmed beyond reasonable doubt that excess childhood morbidity and mortality is attributable to classic vitamin A deficiency, a condition prevalent throughout the developing world.[1] From the relative disbelief that accompanied the first modern description of the association between deficiency and increased childhood mortality,[2] to the ability of intervention programs to reverse it,[3] improvement in the vitamin A status of deficient populations has become a mainstream international health goal.[4,5] Two decades ago, less than six countries were concerned about the problem. Now, over 60 countries worldwide have established plans or programs to address vitamin A deficiency, including the United States.[6]

Despite periodic reports earlier in this century that vitamin A deficiency affected growth, measles mortality, and potentially a host of other health outcomes, physicians largely focused on the dramatic ocular changes ("xerophthalmia"). The earliest of these ocular changes, nightblindness and Bitot's spots, virtually defined "mild deficiency" and the biochemical criteria associated with it.[7] This conceptual framework has now been completely revised: ocular abnormalities reflect moderate to severe deficiency, with clinically relevant implications arising earlier in the process and at milder degrees of impaired vitamin A status.[1]

Childhood mortality rates increase in direct proportion to the severity of deficiency,[2] beginning before ocular changes are clinically apparent.[1] Indeed, among populations where xerophthalmia is rare or absent,[8] this is a fact consistent with the high prevalence of milder manifestations of physiologic disturbance.[1,9] Meta-analyses of community-based prophylaxis trials indicate that improvement in vitamin A status reduces preschool-age (6 mo to 6 y) mortality by 20 to 70%, depending upon the severity of underlying deficiency, the degree to which vitamin A status was improved, and the relative contribution of underlying diseases to all-cause mortality.[1,10]

Most childhood mortality associated with vitamin A deficiency appears to be related to the severity and not the incidence of infectious disease,[8] particularly chronic diarrhea and measles.[1] Indeed, treatment of moderate to severe measles reduces the severity of complications and cuts mortality in half,[11–13] the same degree of measles mortality reduction associated with community-based prophylaxis.[1] Since treatment of measles with high-dose vitamin A produces the same degree of reduction in measles-associated mortality as vitamin A administered prophylactically in community settings, it is likely that the accompanying reduction in deaths, in both instances, is attributable to improvement in vitamin A status.

The mechanism(s) by which vitamin A status influences the severity of infectious diseases is undoubtedly complex, involving the barrier function of epithelial linings (altered early in deficiency) as well as humoral and cell-mediated immunity.[14,15]

Vitamin A also affects differentiation and development of a host of tissues and organs, which probably accounts for its role in immunity, growth, xerophthalmia, and iron metabolism (and associated anemia).[16] That this long-known vitamin, first discovered in 1913, should have such profound and multifaceted effects is no longer surprising: vitamin A regulates the expression of over 300 genes, many of which respond within 1 h to oral administration of vitamin A.

It is now estimated that vitamin A deficiency accounts for 1 to 3 million childhood deaths, 5 to 10 million cases of xerophthalmia, and half a million children being blinded each year.[17] The impact of deficiency on morbidity and mortality among older children and adults, particularly anemia and chronic infectious diseases, is as yet unquantified. Recent interest has focused on the potential role of vitamin A status in modulating the effects of HIV infection, particularly vertical transmission from mother to infant.[18]

Many unresolved scientific questions remain, particularly the role and clinical impact of varying levels of vitamin A status, especially "mild" deficiency (e.g., serum vitamin A of 20–30 μg/dL), a condition that only a decade ago was considered to be within "normal" limits.

The most pressing health issue, however, is to act upon knowledge already at hand: finding innovative ways to improve the vitamin A status of deficient populations. This will require local political commitment and a practical combination of intervention strategies appropriate to the local determinants of deficiency, including cultural preferences, environment, and resources available within the target population(s). Successful interventions usually include some combination of education and social marketing (to increase intake of vitamin A-rich foods), fortification of a com-

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monly consumed item, and/or periodic supplementation. Vitamin A deficiency has virtually disappeared from Singapore, Malaysia, and large areas of China, concomitant with dramatic improvements in economic status. Indonesia radically reduced the prevalence of xerophthalmia, if not the eradication of milder deficiency, through dedicated employment of a combination of programs, moving initially from dependence on costly vertically targeted approaches to ones that were integrated into an expanding primary health infrastructure.[19] With dedication and leadership, the same can occur elsewhere.

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Effects of Vitamin A on Growth of Vitamin A-Deficient Children: Field Studies in Nepal[1,2,3]

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ABSTRACT Inconsistencies have been observed in the impact of vitamin A (VA) supplementation on early child growth. To help clarify this issue, a cohort of 3377 rural Nepalese, nonxerophthalmic children 12–60 mo of age were randomized by ward to receive vitamin A [60,000 μg retinol equivalents (RE)] or placebo-control (300 RE) supplementation once every 4 mo and followed for 16 mo. VA had no impact on annual weight gain or linear growth. However, arm circumference (AC) and muscle area (MA) growth improved in VA recipients, by 0.13 cm and 25 mm^2, respectively, over controls. Growth of children with xerophthalmia, who were treated with $\geq$ 120,000 RE at base line, was also compared to that of nonxerophthalmic children, stratified by initial wasting status, and adjusted for sex, baseline age and measurement status. Among initially nonwasted children (AC $\geq$ 13.5 cm), VA-treated xerophthalmic children (n = 86) gained 0.7 cm more in linear growth than nonxerophthalmic children. Among initially wasted children (AC < 13.5 cm), VA-treated children (n = 34) gained additional weight (672 g), height (~1 cm), muscle (76 mm^2) and fat (79 mm^2) areas, and subscapular skinfold (1.3 mm) compared to changes observed in nonxerophthalmic children. Relative increments in soft tissue growth occurred within 4 mo of VA treatment, while the effect on linear growth was gradual. Moderate-to-severe VA deficiency, marked by xerophthalmia, is likely to impair normal physical growth, but milder stages of deficiency may not have this effect in rural South Asia. J. Nutr. 127: 1957–1965, 1997.

KEY WORDS: • *vitamin A deficiency* • *xerophthalmia* • *growth* • *community trial* • *children*

Vitamin A deficiency is an important child health problem in many developing countries, with consequences ranging from potentially blinding xerophthalmia to increased risks of infection and mortality (Sommer and West 1996). Increased vitamin A intake, achieved by supplementation or fortification of food stuffs, confers a clear survival benefit to young children (Beaton et al. 1993, Sommer and West 1996).

Vitamin A is an essential nutrient for mammalian growth (McCollum and Davis 1913) but it has been difficult to demonstrate the effect of vitamin A deficiency on the growth of children. The motivation to verify an effect of vitamin A deficiency on growth and hence extend the assertion of McCollum and Davis to the human, is less to influence public policy (since aims to prevent childhood blindness and mortality suffice in this regard) than to understand the sum of health effects that can be attributed to vitamin A deficiency and its prevention in populations where other nutritional deficiencies, infection and mortality are common in children. It is the working hypothesis of this paper that moderate-to-severe vitamin A deficiency, marked by the presence of night blindness or clinical eye signs, can and does impair physical growth in young children compared to the growth of generally wasted and stunted children who are also likely to be deficient in vitamin A, but lack eye signs of xerophthalmia.

Clinical vitamin A deficiency has long been linked to poor child growth on a cross-sectional basis; often the more severe the eye signs, the more severe the stunting and wasting (Sommer 1982). Children who develop mild xerophthalmia (night blindness or Bitot's spots) also show less weight gain and linear growth than their nonxerophthalmic peers. Conversely, improved weight gain can accompany spontaneous recovery from xerophthalmia, although catch-up linear growth is less evident

[1] This study was carried out under Cooperative Agreement No. DAN 0045-A-00-5094-00 between the Office of Health and Nutrition, U.S. Agency for International Development (USAID), Washington, DC and the CHN/DCPO, The Johns Hopkins University, Baltimore, MD, USA, 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.

[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] The Sarlahi Study Group (in addition to the authors) include Drs. R.P. Pokhrel, B.D. Chataut, D. Calder, J. Gmunder, J. Humphrey, J. Tielsch, H. Taylor, M.R. Pandey; Mr. D. Piet, J. Canner, N.N. Achariya, D.N. Mandal, T.R. Sakya, B.B. Shrestha and R.K. Shrestha.

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(Tarwotjo et al. 1992). These associations may represent a direct effect of vitamin A nutriture on growth or they may be indirect, mediated by other co-varying nutritional and morbid states that can effect growth. Vitamin A supplementation trials have produced mixed results, ranging from improved ponderal (West et al. 1988) or linear (Muhilal et al. 1988) growth to little or no discernable effects (Brown et al. 1980, Fawzi et al. 1997, Lie et al. 1993, Rahmathullah et al. 1991, Ramakrishnan et al. 1995) in nonxerophthalmic children, casting doubt about the importance of vitamin A as a determinant of child growth in free-living populations (Ramakrishnan et al. 1995).

Here we report the impact of periodic vitamin A supplementation on ponderal, linear and body compositional growth of nonxerophthalmic children participating in a randomized, double-masked community trial. We also explore the hypothesis that vitamin A repletion of moderately-to-severely deficient children, marked by the presence of xerophthalmia, can improve growth under rural conditions where other forms of childhood malnutrition, infection and risk of mortality are moderately severe and widespread.

METHODS

The impact on growth of high-potency vitamin A supplementation once every 4 mo was assessed over a 16-mo period in a randomly sampled cohort of preschool-aged children participating in a double-masked, randomized, placebo-controlled community trial in the terai (plains) district of Sarlahi, Nepal. Field work was conducted between September, 1989 and December, 1991. The design (West et al. 1991), procedures (Pradhan et al. 1994) and findings of the trial related to the impact of vitamin A on mortality (Pokhrel et al. 1994, West. et al. 1991 and 1995) and xerophthalmia (Katz et al. 1995) have been reported. We have also attempted to assess the growth impact of vitamin A on moderately-to-severely vitamin A–deficient children by comparing within each supplement group the growth responses of children with xerophthalmia, who were treated with vitamin A at base line irrespective of their random assignment, with the growth of nonxerophthalmic children, adjusting for multiple potential confounders.

Briefly, 261 wards in Sarlahi District were randomly assigned to vitamin A (n = 131) or control (n = 130) supplement status. Twenty wards were randomly sampled from each of these two groups to investigate the impact of vitamin A on growth. This analysis is restricted to a cohort of children 12–60 mo of age at the outset (the age range for xerophthalmia in this study). At base line, children were enumerated and dosed with ward-assigned, coded capsules of identical appearance containing either 60,000 μg retinal equivalents (RE)[5] (200,000 IU "vitamin A") or 300 μg RE (1000 IU) ("control") of vitamin A as retinyl palmitate, plus 40 IU of vitamin E (a gift of Roche, Basel, Switzerland). Seven-day morbidity histories were obtained. Parents or guardians brought their children to a central site for ocular examination and nutritional assessment. Xerophthalmia was diagnosed by standard, clinical criteria (Sommer 1995) and by probing for a history of nightblindness using a local term (*rataundhi*) (Khatry et al. 1995). Cases in both groups were treated with 120,000 μg RE of vitamin A (one capsule at presentation and a second taken the next day) and referred to local health posts for clinical follow-up and further treatment, if indicated. Masking was preserved for the purpose of the trial. Thus, children in the vitamin A group received a (coded) vitamin A supplement in their home just prior to examination, providing them with a total dose of 180,000 μg RE. Children in the control group received their assigned placebo supplement at home. Children continued to be visited at home every 4 mo and dosed according to their ward allocation in the trial. This design produced four supplement-clinical status groups: nonxerophthalmic children receiving vitamin A or placebo-control supplements every 4 mo and children with xerophthalmia at base line in each of these two groups (**Fig. 1**). Having all received at least 120,000 μg RE at the outset, cases of xerophthalmia in both supplement groups have been pooled for the growth analysis presented here, except where noted.

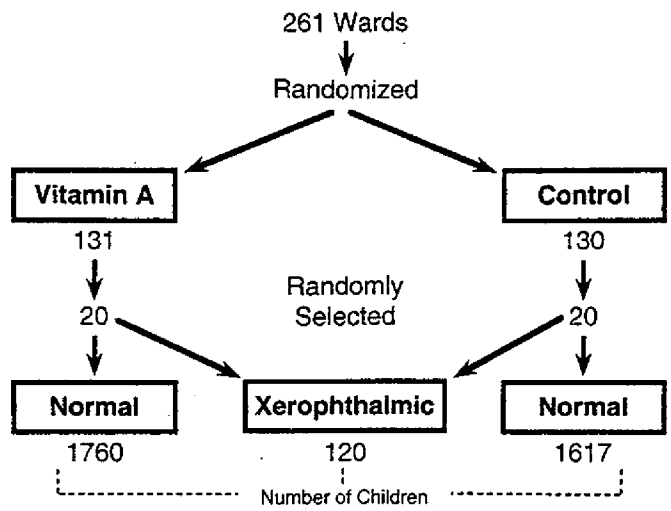

FIGURE 1 Study design and number of wards and children enrolled in the growth cohort, by supplement group and clinical status at baseline.

Baseline anthropometry included *1*) weight (WT) measured when children were naked or lightly clad, read to the nearest 0.1 kg on a hanging spring scale (Salter Ltd, UK) and calibrated daily against known, standard weights; *2*) standing height (≥ 24 mo) or recumbent length (12–23 mo) (HT), read to the nearest 0.1 cm on a steel tape attached to a wooden board with a foot-plate and sliding head block (Shorr Productions, Woonsocket, RI); *3*) left mid-upper arm circumference (AC), read to the nearest 0.1 cm using a Zerfas insertion tape (Zerfas 1975); and *4*) skinfold thickness, obtained to the nearest 0.1 mm after 2–4 seconds' application of a caliper to the left tricipital (TS) and subscapular (SS) sites (Holtain Ltd, Crosswell, Crymch, Wales, UK). The median of 3 or 5 independent readings was recorded for each measurement, except for weight which was usually read once after the pointer on the scale was steady for ≥2 s. Measurements were performed by a team of four trained anthropometrists: one observer-assistant pair assigned to measure WT and HT and a second pair to measure AC and skinfolds.

Household visits and anthropometry at the central site (WT, HT and AC) were repeated every 4 mo for 16 mo (5 visits) by the same team. Skinfolds (TS and SS) were remeasured only at the 16-mo visit, the final visit during which children of this age were in the trial (Pokhrel et al. 1994).

Intrateam measurement error was monitored by independently repeating all anthropometry 1–2 h later in a random 5% sample of children at the central site during each visit (n = 411–414 replicates for WT, HT and AC and n = 75 for TS and SS). The mean technical error, expressed as a standard deviation (SD = $\sqrt{\Sigma d^2/2n}$, where d = the difference between paired measurements and n = the number of children) was 76 g for WT, 0.35 cm for HT, 0.14 cm for AC, 0.34 mm for TS and 0.27 mm for SS.

In addition to actual measurements, estimates of cross-sectional upper arm muscle (plus humoral bone) (MA) and fat (FA) areas as indicators of apparent body muscle and subcutaneous fat mass (Gibson 1990), respectively, were derived from AC and TS measurements by standard formulas (Frisancho 1981). Weight-for-height, height-for-age and weight-for-age values were expressed as standard normal deviates (Z-scores, or WHZ, HAZ and WAZ, respectively) based on the National Center for Health Statistics, median age-sex-specific values for American children (Hamill et al. 1977).

Group differences at base line were evaluated by χ^2 for discreet variables and analysis of variance for continuous variables. Differences

[5] Abbreviations used: AC, arm circumference; CL, 95% confidence limits; FA, fat area; HAZ, height-for-age Z-score; HT, height; MA, muscle area; RE, retinol equivalent; SS, subscapular skinfold; TS; tricipital skinfold; VA, vitamin A; WAZ, weight-for-age Z-score; WHZ, weight-for-height Z-score; WT, weight.

in 4-, 8-, 12- and 16-mo growth increments were compared between 1) randomized groups of nonxerophthalmic children and 2) xerophthalmic and nonxerophthalmic children, by multivariable linear regression adjusted for age (in mo), the baseline value of the specific measurement or indicator being compared, and sex of the child (Kleinbaum and Kupper 1978). Beta-coefficients provided estimates of the adjusted cumulative growth effects of vitamin A supplementation relative to placebo receipt in the randomized groups. For comparisons between xerophthalmic and nonxerophthalmic children, the beta-coefficients represent the incremental growth of vitamin A–treated children with xerophthalmia over (or under) the growth rate of nonxerophthalmic children. It became apparent during analysis that, in this population, arm circumference (AC) size was an important effect modifier for observed growth responses to vitamin A. Therefore, growth comparisons were stratified by initial AC size, < 13.5 and ≥ 13.5 cm, representing children who were wasted and not wasted, respectively, at the outset. Ninety-five percent confidence limits (CL) for relative increments were derived from the standard errors (SEM) of the regression coefficients.

Additional regression analyses were carried out to compare growth increments between randomized groups and between xerophthalmic and nonxerophthalmic children, adjusting for other potential influences such as baseline morbidity (using diarrhea defined as ≥1 day of ≥4 loose watery stools the previous week as a proxy), breastfeeding status at the outset (visit 1) and at the relevant visit ending an interval (yes vs. no), and education of the head of household (any vs. none) as a proxy for socioeconomic status. These adjustments had no significant or discernable effects on growth outcomes, but decreased the effective sample size by about one-third for each comparison due to missing data obtained either by design (e.g., morbidity data were only collected for children who were under parental observation the entire previous week) or due to non-responses on some of these variables. Thus, only findings adjusted by age, baseline status and sex are presented to preserve sample size and power.

Participation of families and their children in the study was voluntary. Written and verbal disclosures were made about the study to participating communities and households, respectively, and study procedures were approved by the Nepal Health Research Council, Kathmandu, Nepal and the Joint Committee on Clinical Investigation, the Johns Hopkins School of Medicine, Baltimore, MD, USA.

RESULTS

Baseline comparisons. A total of 3811 children 12–60 mo of age were recruited at base line (n = 1991 in the vitamin A group; n = 1820 in the control group). Of these, 3497 (92% of all eligible; 91% in the vitamin A group and 93% of controls) obtained an eye exam and anthropometry at a central site (**Table 1**): 1760 nonxerophthalmic and 52 xerophthalmic children in the vitamin A group and 1617 nonxerophthalmic and 68 xerophthalmic children in the control group. These children constitute the present growth cohort. Of all clinically normal children in both supplement groups assessed at base line, 87–91% were remeasured at 4, 8 and 12 mo; 84% in both groups were remeasured at 16 mo. Comparable follow-up was achieved for xerophthalmic children with ~80% followed to completion. Follow-up was similar by gender (not shown) and initial wasting status (Table 1).

Nonxerophthalmic children in the two randomized groups were comparable except for a 0.21 cm larger AC (and larger constituent MA) among controls (**Table 2**). Xerophthalmic children in the two supplement groups were similar in all baseline characteristics (not shown). However, xerophthalmic children were older (by ~7 mo), less likely to be breastfeeding (15 vs. ~42%), and came from more socioeconomically deprived homes than nonxerophthalmic children (Table 2) (Khatry et al. 1995).

Baseline anthropometric profiles of nonxerophthalmic and xerophthalmic children were compared between and within each AC stratum. Wasted children, with an AC < 13.5 cm, had lower WHZ, WAZ and HAZ scores (**Fig. 2A**), smaller SS and AC values (Fig. 2B) and less MA and FA (Fig. 2C) than children who were not wasted (all $P < 0.0001$). For all indicators, nonwasted xerophthalmic and nonxerophthalmic children were comparable. However, wasted children with xerophthalmia were still more stunted and acutely malnourished than nonxerophthalmic children with an AC < 13.5 cm, significantly so for HAZ, WAZ, SS, AC and MA (all $P < 0.01$) and FA ($P < 0.03$). Also, eye signs of xerophthalmia tended to be more severe in wasted than nonwasted children (66% vs. 55% with Bitot's spots, 2% vs. 0% with corneal xerosis, respectively).

Growth effects in nonxerophthalmic children. Findings from the randomized trial show that four months after initial dosing (after the major rice harvest in November and December) vitamin A–dosed children gained 121 g less weight [and 0.11 less in WHZ (95% CL: −0.16, −0.07)] than controls; the effect was comparable by baseline AC status (**Table 3**). This relative decrement was offset by relative weight gain over the next 8 mo (encompassing the late dry and early monsoon season) such that, after 12 mo, vitamin A recipients had gained an average of 38 g more than controls (not significant). After 16 mo (and a second peri-harvest interval) children receiving vitamin A had again gained less total weight than controls (−72 g, −0.06 WHZ, 95% CL: −0.10, −0.02). Differences between groups in weight gain were comparable by baseline AC (Table 3), sex and age (not shown).

There was no effect of vitamin A on linear growth (0.05 cm and 0.01 HAZ at 16 mo), except for a small increase of 0.13 cm after 12 mo among nonwasted vitamin A recipients (Table 3). Arm circumferential growth of vitamin A recipients

TABLE 1

Numbers of children receiving anthropometry by visit, supplement-clinical status and initial arm circumference[1]

Visit	Nonxerophthalmic children: Vitamin A	Nonxerophthalmic children: Control	Xerophthalmic children	Total
		n		
Baseline	1760	1617	120	3497
	(100%)	(100%)	(100%)	(100%)
<13.5 cm	681	506	34	1221
≥13.5 cm	1078	1109	86	2273
4 months	1592	1450	104	3150
	(90%)	(90%)	(87%)	(90%)
<13.5 cm	617	445	30	1092
≥13.5 cm	975	1005	74	2054
8 months	1599	1434	98	3139
	(91%)	(89%)	(82%)	(90%)
<13.5 cm	620	438	27	1085
≥13.5 cm	979	996	71	2046
12 months	1578	1405	100	3095
	(90%)	(87%)	(83%)	(89%)
<13.5 cm	604	429	26	1059
≥13.5 cm	974	976	74	2024
16 months	1481	1361	96	2954
	(84%)	(84%)	(80%)	(84%)
<13.5 cm	587	439	25	1051
≥13.5 cm	894	922	71	1887

[1] Maximum number measured at each follow-up visit. Maximum number of missing values for one or more measurements at 4, 8, 12 and 16 mo for the nonxerophthalmic vitamin A group = 5, 4, 26 and control group = 5, 2, 19 and 5; and 0, 1, 0 and 0 for all xerophthalmic children.

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TABLE 2

Baseline child and household characteristics (at visit 1) by randomized supplement group and xerophthalmia status

Characteristic	Nonxerophthalmic children		Xerophthalmic children
	Vitamin A	Control	
Child			
n	1760	1617	120
Age,[1] *mo*	35 ± 14	35 ± 14	42 ± 11[5]
Male, %	52.9	52.5	55.0
Breastfed, %	42.2	43.1	15.0[5]
Nutritional status[1,2]			
WT, *kg*	10.83 ± 2.52	10.81 ± 2.49	11.50 ± 2.43[4]
HT, *cm*	84.3 ± 9.9	84.4 ± 9.9	88.0 ± 9.10[5]
HAZ	−2.46 ± 1.25	−2.44 ± 1.25	−2.63 ± 1.50
WAZ	−2.23 ± 0.95	−2.25 ± 0.95	−2.36 ± 1.89
WHZ	−1.01 ± 0.82	−1.05 ± 0.82	−1.11 ± 0.89
AC, *cm*	13.71 ± 1.13[3]	13.92 ± 1.14	13.77 ± 1.41
MA,[2] *mm*	1066 ± 182[3]	1104 ± 181	1087 ± 210
FA,[2] *mm*	440 ± 111	449 ± 108	437 ± 127
SS, *mm*	5.8 ± 1.3	5.8 ± 1.3	5.7 ± 1.6
Morbidity past week, %			
Diarrhea	12.2	10.8	10.0
Cough	16.1	15.1	20.8
Fever	15.5	18.2	22.5
Household			
Mother			
n	1295	1186	114
Age,[1] *y*	28 ± 7	28 ± 6	29 ± 6
Any education, %	8.3	7.8	2.6[4]
Head			
n	1234	1123	108
Any education, %	33.1	31.6	17.6[5]
Laborer, %	22.1	19.5	39.8[5]

[1] Mean ± standard deviation.
[2] WT = weight; HT = height; HAZ, WAZ, WHZ = z-scores for HT-for-age, WT-for-age and WT-for-HT, respectively; AC = (left) upper arm circumference; MA, FA = (left) upper arm muscle and fat areas, respectively; SS = (left) subcapsular skinfold
[3] Difference between vitamin A and control children, $P < 0.001$
[4] Difference between xerophthalmic and all nonxerophthalmic children/households, $P < 0.05$.
[5] Difference between xerophthalmic and all nonxerophthalmic children/households, $P \leq 0.001$.

increased 0.16 cm over that of controls in the first 4 mo, more apparent in nonwasted children. This increment, which persisted, was associated with a 25 mm^2 (or 32%) increase in arm MA over the growth in MA of nonxerophthalmic children (**Table 4**) which had increased by a total of 77 mm^2 (mean) over the 16-mo period (not shown). No differences between groups were observed in rates of change in FA and SS (Table 4).

Growth of xerophthalmic vs. nonxerophthalmic children. The apparent ponderal growth response of xerophthalmic children to vitamin A, analyzed outside the randomized design, differed markedly by initial wasting status, whereas the linear growth response was more uniform across AC strata, compared to their nonxerophthalmic peers (**Table 5**).

Wasted xerophthalmic children gained 644 g more in weight during the first 4 mo than nonxerophthalmic, wasted children, representing an 84% increase over the mean 4-month gain of 765 g in the latter group. The initial increment was accompanied by relative improvement in weight-for-height, by 0.57 WHZ (95% CL: 0.37, 0.77), reflecting early, preferential gain in soft tissue. This weight increment persisted for the duration of the study. Relative change in AC growth paralleled that of weight gain: there was an initial increase of 0.77 cm in arm circumferential growth of wasted xerophthalmic children compared to wasted nonxerophthalmic children. The increment persisted through the 16-month follow-up (0.71 cm), by which time wasted xerophthalmics showed additional gains in MA of 76 mm^2 and FA of 79 mm^2 over arm area gains in other initially wasted children (Table 4). Subscapular skinfolds also preferentially increased in wasted-xerophthalmics, by 1.31 mm. Linear growth (ΔHT in Table 5) of wasted xerophthalmics gradually accelerated over that of wasted nonxerophthalmic children, showing cumulative relative increments of 0.20, 0.49, 0.74 and 0.97 cm by 16 mo, representing a 10% increase over the mean linear growth (of 9.8 cm) in the latter group over this time.

Vitamin A treatment was associated with modest, gradual weight gain (196 g by 16 mo) in initially nonwasted xerophthalmics (AC $\geq$ 13.5 cm) compared to the weight gain of their nonwasted, nonxerophthalmic peers (Table 5). Linear growth of xerophthalmics also improved, by 0.69 cm after 16 mo, representing 8% of the mean gain in height (of 9.2 cm) of nonxerophthalmic children. However, nonwasted xerophthalmics showed no advantage in changed weight-for-height (with relative increments varying from −0.04 to −0.01 WHZ, all not significant), AC (Table 5), MA, FA or SS (Table 4), indicating that the relative gain in weight was pondostatural. Supporting this interpretation, the 196 g weight gain advantage of VA-treated xerophthalmics was no longer evident once ΔHT was introduced into the regression equation (b = 70 g)

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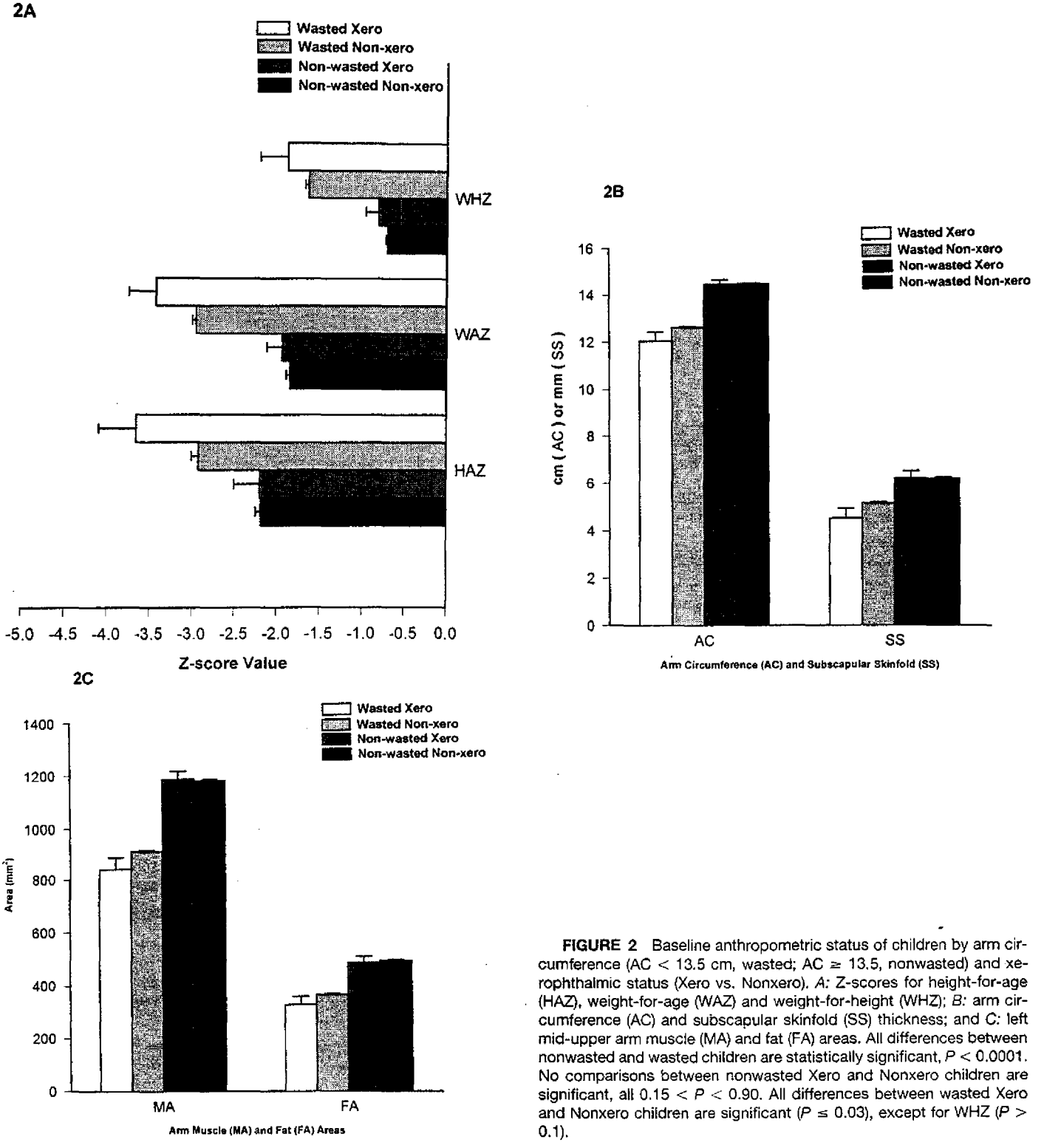

FIGURE 2 Baseline anthropometric status of children by arm circumference (AC < 13.5 cm, wasted; AC ≥ 13.5, nonwasted) and xerophthalmic status (Xero vs. Nonxero). *A:* Z-scores for height-for-age (HAZ), weight-for-age (WAZ) and weight-for-height (WHZ); *B:* arm circumference (AC) and subscapular skinfold (SS) thickness; and *C:* left mid-upper arm muscle (MA) and fat (FA) areas. All differences between nonwasted and wasted children are statistically significant, $P < 0.0001$. No comparisons between nonwasted Xero and Nonxero children are significant, all $0.15 < P < 0.90$. All differences between wasted Xero and Nonxero children are significant ($P \leq 0.03$), except for WHZ ($P > 0.1$).

containing age, sex, baseline WT and other covariates suggesting that the differential in weight gain was largely due to a difference in height gain between groups.

Adding an interaction term in each regression for xerophthalmia × AC showed highly significant interactions ($P < 0.001$ for all) for ΔWT, ΔWAZ, ΔWHZ and ΔAC and insignificant interactions for ΔHT and ΔHAZ ($P > 0.4$ for all) during each cumulative interval, and strong interactions ($P < 0.01$ for all) for 16-mo differences in MA, FA and SS, as Tables 5 and 6, respectively, indicate. The size of interaction between effects in xerophthalmic children with a low vs. higher AC can be estimated by subtracting the coefficient values for children with AC > 13.5 cm from values for children with AC < 13.5 cm (e.g., 621 g difference in weight gain between AC groups from 0–4 mo).

Also, adding a xerophthalmia × supplement group interaction to each regression showed that continuously VA-supplemented xerophthalmics gained more fat by 16 mo than cases

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TABLE 3

Cumulative differences in growth by interval and baseline arm circumference (AC) between nonxerophthalmic children, 12–60 mo of age at baseline, randomized to vitamin A or placebo control supplements every four months[1]

	Δ WT[2]		Δ HT		Δ AC	
Follow-up interval	b	95% CL[3]	b	95% CL[3]	b	95% CL[3]
	g		*cm*		*cm*	
0–4 months	−121	(−159, −93)	0.02	(−0.04, 0.08)	0.16	(0.12, 0.20)
AC < 13.5 cm	−123	(−190, −56)	0.05	(−0.09, 0.19)	0.09	(0.01, 0.17)
AC ≥ 13.5 cm	−108	(−155, −61)	0.03	(−0.05, 0.11)	0.20	(0.16, 0.24)
0–8 months	−53	(−73, −33)	0.00	(−0.08, 0.08)	0.10	(0.06, 0.14)
AC < 13.5 cm	−32	(−103, 39)	−0.06	(−0.22, 0.10)	0.07	(−0.01, 0.15)
AC ≥ 13.5 cm	−60	(−109, −12)	0.06	(−0.04, 0.16)	0.12	(0.09, 0.17)
0–12 months	38	(−4, −70)	0.07	(−0.03, 0.17)	0.23	(0.19, 0.27)
AC < 13.5 cm	48	(−28, 124)	0.00	(−0.20, 0.20)	0.22	(0.14, 0.30)
AC ≥ 13.5 cm	24	(−29, 77)	0.13	(0.01, 0.25)	0.23	(0.17, 0.29)
0–16 months	−72	(−125, −19)	0.05	(−0.07, 0.17)	0.13	(0.09, 0.17)
AC < 13.5 cm	−80	(−172, 12)	0.00	(−0.23, 0.23)	0.07	(−0.03, 0.17)
AC ≥ 13.5 cm	−64	(−127, −1)	0.11	(−0.03, 0.25)	0.16	(0.10, 0.22)

[1] Differences obtained from multiple linear regression coefficients (b) representing the cumulative effect of vitamin A (60,000 μg RE) vs. placebo control (300 μg RE) supplementation every 4 mo in nonxerophthalmic children adjusted for age and the specified measurement value at baseline and sex.

[2] WT = weight, HT = height, AC = arm circumference.

[3] 95% confidence limits of b derived from standard errors of regression coefficients.

treated with VA only at baseline: AC increased by an additional 0.30 cm (95% CL: 0.02, 0.58), FA increased by 38 mm^2 (−2, 78) and SS increased by 0.42 mm (−0.05, 0.89), while the relative increase in MA was less and not significant [29 mm^2 (95% CL: −11, 69)].

Differences in WAZ growth (Figure 3) show that treatment of xerophthalmic children with vitamin A favored the growth of wasted xerophthalmic vs. wasted nonxerophthalmic children, irrespective of age. Within 4 mo, WAZ growth accelerated in wasted xerophthalmics by ~0.4 Z-scores over that of wasted nonxerophthalmic children; thus, identifying a subgroup of initially more malnourished, vitamin A–deficient children whose growth responded to vitamin A. Among nonwasted children, vitamin A treatment conferred no WAZ growth advantage to xerophthalmics over their similarly nourished, nonxerophthalmic peers, except for a 0.11 Z increase in WAZ in older xerophthalmics (36–60 mo of age at base line) after 16 mo.

DISCUSSION

Nonxerophthalmic children. Findings from the randomized trial show that periodic, high-potency vitamin A receipt exerted no effect on linear growth or weight gain over the course of an entire year in rural Nepal. However, ponderal growth was negatively affected by vitamin A during two consecutive periharvest seasons, reflected by ~120 g reductions in weight gain per 4 mo compared to placebo-control children. It was accompanied each year by small relative decreases in weight-for-height suggesting that vitamin A recipients may have been accumulating less fat than children not receiving vitamin A. Mechanisms for this effect remain to be explained. However, this seasonal decline in weight gain velocity was countered by improved weight gain over the remaining eight months of the year, when food availability and anthropometric status typically decline and morbidity increases (K. West et al., unpublished data), leaving no net effect of vitamin A on

TABLE 4

Differences in sixteen-month change in muscle and fat indicators by baseline arm circumference (AC) in children 12–60 mo of age at baseline[1]

	Comparisons of nonxerophthalmic children vitamin A vs. control		Xerophthalmic vs. nonxerophthalmic comparisons	
Indicator	b	95% CL	b	95% CL
Δ Muscle area, *mm²*	25	(17, 33)	35	(15, 55)
AC < 13.5 cm	20	(8, 32)	76	(35, 117)
AC ≥ 13.5 cm	28	(19, 37)	16	(−8, 40)
Δ Fat area, *mm²*	0	(−8, 8)	7	(−13, 27)
AC < 13.5 cm	−5	(−17, 17)	79	(40, 118)
AC ≥ 13.5 cm	4	(−5, 13)	−22	(−46, 2)
Δ Subcapsular skinfold, *mm*	0	(−08, 0.08)	0.36	(0.13, 0.60)
AC < 13.5 cm	−0.05	(−0.19, 0.09)	1.31	(0.86, 1.76)
AC ≥ 13.5 cm	0.05	(−0.05, 0.15)	−0.02	(−0.29, 0.25)

[1] Differences obtained from multiple linear regression coefficients (b) representing the growth effects with 95% confidence limits (95% CL).

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TABLE 5

Cumulative differences in growth by interval and initial arm circumference (AC) between xerophthalmic and nonxerophthalmic children, 12–60 mo of age at baseline[1]

	Δ WT		Δ HT		Δ AC	
Follow-up interval	b	(95% CL)[2]	b	(95% CL)	b	(95% CL)
	g		*cm*		*cm*	
0–4 months	221	(115, 327)	0.21	(0.03, 0.39)	0.18	(0.06, 0.30)
AC < 13.5 cm	644	(440, 848)	0.20	(−0.19, 0.59)	0.77	(0.54, 1.01)
AC ≥ 13.5 cm	23	(−100, 146)	0.22	(0.02, 0.42)	−0.09	(−0.23, 0.05)
0–8 months	231	(117, 345)	0.34	(0.11, 0.58)	0.23	(0.09, 0.37)
AC < 13.5 cm	686	(459, 913)	0.49	(−0.04, 1.02)	0.79	(0.52, 1.06)
AC ≥ 13.5 cm	37	(−92, 166)	0.29	(0.04, 0.55)	−0.01	(−0.15, 0.13)
0–12 months	276	(153, 399)	0.51	(0.24, 0.78)	0.19	(0.05, 0.33)
AC < 13.5 cm	725	(472, 978)	0.74	(0.13, 1.35)	0.74	(0.45, 1.03)
AC ≥ 13.5 cm	106	(−33, 245)	0.44	(0.15, 0.73)	−0.03	(−0.17, 0.11)
0–16 months	343	(194, 492)	0.77	(0.44, 1.10)	0.20	(0.06, 0.34)
AC < 13.5 cm	672	(362, 982)	0.97	(0.23, 1.72)	0.71	(0.40, 1.02)
AC ≥ 13.5 cm	196	(31, 361)	0.69	(0.34, 1.04)	−0.02	(−0.16, 0.12)

[1] Differences obtained from multiple linear regression coefficients (b) representing the cumulative effect of vitamin A treatment (either 120,000 μg RE or 180,000 μg RE at baseline) of xerophthalmic children on growth compared to growth of nonxerophthalmic children (periodically receiving vitamin A or placebo), adjusted for age, measurement status at baseline and sex.

[2] 95% confidence limits of b derived from standard errors of regression coefficients.

annual weight gain. Seasonal increases in weight gain velocity associated with vitamin A receipt have only recently been appreciated. Indian children randomized to receive high-potency vitamin A between April and July (late hot/early rainy seasons) also showed significantly greater weight gain (140 g) and less wasting malnutrition than placebo controls dosed in the same season, but no effects were observed the rest of the year (Bahl et al. 1997).

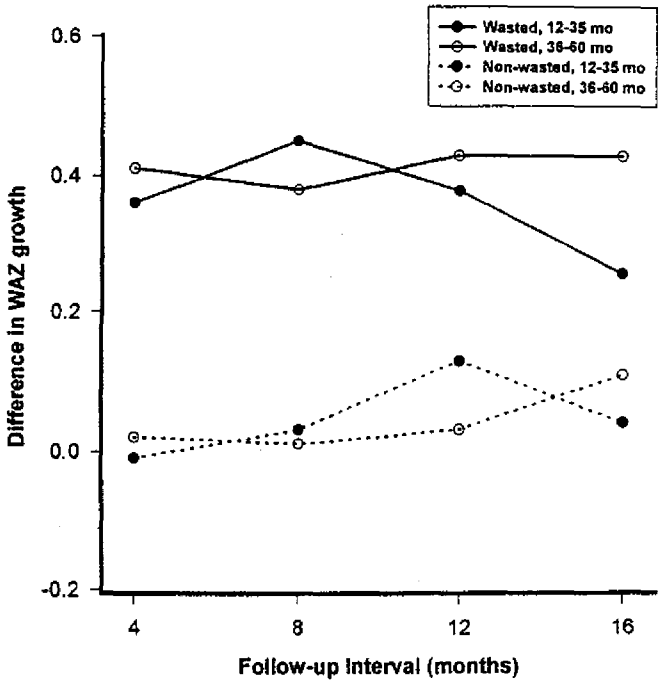

FIGURE 3 Growth by weight-for-age (WAZ) of children with xerophthalmia compared to children without xerophthalmia, supplement groups combined, by wasting status and age at baseline and adjusted for sex, age (in months) and baseline WAZ by linear regression. All differences in Δ WAZ between wasted children and wasted nonxerophthalmic children are significant ($P \leq 0.005$) except for 12–35 mo-olds after 12 mo ($P \leq 0.02$) and 16 mo ($P = 0.12$). Only the WAZ growth of nonwasted children after 16 months is significantly different from nonwasted nonxerophthalmic children ($P \leq 0.01$).

The lack of impact of vitamin A on annual ponderal or linear growth is consistent with findings from two other trials among nonxerophthalmic children in India where oral vitamin A, provided as a weekly low dose to children ~5 to 71 mo of age (Rahmathullah et al. 1991) or as a periodic, high-potency supplement to children 6 to 36 mo of age (Ramakrishnan et al. 1995), failed to markedly influence 12-mo weight gain or linear growth, but seasonality was not examined. Thus, it appears that in South Asia, where moderate stunting and wasting (ACC/SCN Report, 1992) typically coexist with multiple micronutrient deficiencies (Gopalan 1989), vitamin A supplementation alone may have little effect on height or weight gain of most preschool children. This is in contrast to studies in Southeast Asia where improved weight gain (West et al. 1988) and linear growth (Muhilal et al. 1988) were observed in vitamin A–supplemented children in populations that were generally in better health and better nourished than the population in rural South Asia (UNICEF, 1997).

Vitamin A may have caused a change of body composition, reflected by a 32% relative increase in the growth of upper arm muscle area (~25 mm^2) which contributed to a small but significant increase in arm circumference (0.13 cm) after 16 mo in both wasted and nonwasted VA-supplemented children. Upper arm muscle area is an index of skeletal muscle mass (Gibson 1990), shares high correlations with other indices of lean body mass, such as the creatinine/height ratio (Heymsfield et al. 1982; Trowbridge et al. 1982), and gradually responds to protein-energy deprivation and recovery (Wright and Heymsfield 1984). In the absence of an effect on subcutaneous arm and trunk fat, the change in muscle (and humoral bone) area may have reflected a positive shift in lean body mass with improved vitamin A nutriture. The effect was similar to the 36 mm^2 relative increase in arm muscle area observed after a year of vitamin A supplementation in preschool boys in Indonesia (West et al. 1988). Improved lean body mass, assessed

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in this study by mid-arm anthropometry, may represent an infrequently evaluated aspect of child growth that responds to vitamin A, even in the absence of improved height and weight.

Xerophthalmic children. This trial offered a unique opportunity to examine, outside the original randomized design, the effects of vitamin A on the growth of clinically vitamin A–deficient children, representing ~3% of this child population (Katz et al. 1996; Khatry et al. 1995). Previous vitamin A–growth trials have excluded, either a priori or in the analysis, children with xerophthalmia (Fawzie et al. 1997, Rahmathullah et al. 1991, Ramakrishnan et al. 1995, West et al. 1988) who, being moderately-to-severely vitamin A–deficient (Sommer and West 1996), may have been expected to show a growth response to vitamin A. Mild xerophthalmia has been associated with decelerated linear and ponderal growth (Tarwotjo et al. 1992), low attained height, (Cohen et al. 1986, Khatry et al. 1995, Mele et al. 1991, Santos et al. 1983), mild wasting (Hussain et al. 1996, Khatry et al. 1995, Mele et al. 1991) and increased risks of morbidity (Milton et al. 1987, Sommer et al. 1984) and mortality (Sommer et al. 1983).

Xerophthalmic children who were not wasted at the outset gradually accelerated their linear growth over nonxerophthalmic, nonwasted children, by 0.7 cm, or 10% after 16 mo. Their relative increase in weight gain of ~200 g by the end of the trial was pondostatural; that is, it could be attributed to their improved linear growth since *1*) there was no significant effect of treatment on changes in weight-for-height, arm fat area or subscapular skinfold thickness, and *2*) relative change in height largely explained the increment in weight.

Wasted xerophthalmic children, on the other hand, showed marked increases in weight gain (644 g) and arm circumferential growth (0.7 cm) within four months of being treated with vitamin A, representing 84 and 130% increases, respectively, over growth in these measures by other children who were wasted at the outset. No further improvements in weight gain or arm circumferential growth occurred, while height gain steadily improved over that of other wasted children, reaching a ~1 cm increment after 16 mo (an 8% increase). Rapid weight gain in the first four months likely represented a gain in fat. While not assessed at the second visit, initially wasted children had clearly gained more fat by the end of the study, evident by differential increases in arm fat area and subscapular skinfold thickness. Yet, lean mass also likely improved, reflected by increased arm muscle area growth. By the final round, change in weight-for-height (ΔWHZ) was more highly correlated with age-adjusted change in upper arm muscle area in wasted xerophthalmics ($r = 0.72$) than in other wasted children ($r = 0.43$).

An initial growth response of soft tissue to vitamin A in malnourished, moderately-to-severely vitamin A–deficient children has been reported elsewhere. Rapid ponderal gain was seen in a randomly selected group of mildly wasted, hyporetinemic children with measles in South Africa after being treated with vitamin A (520 g gain in 6 mo), with 75% of the effect occurring in the first 6 wk (Coutsoudis et al. 1991). In Indonesia, children spontaneously recovering from mild xerophthalmia regained all of their calculated weight decrement (~120 g) in a 3-mo period, but not height deficit even after 6 mo (Tarwotjo et al. 1992), suggesting the need for longer follow-up to discern a potential effect of vitamin A on linear growth. However, a more recent randomized, double-masked trial in Indonesia has also shown high-potency vitamin A to stimulate both linear and ponderal growth in severely vitamin A–deficient children (serum retinol < -0.35 μmol/L) (Hadi et al. 1997).

It is possible that improved growth observed in wasted xerophthalmic children may have been due to factors other than vitamin A receipt, such as effective follow-up at primary health care centers or better child care and feeding at home once children were diagnosed. These, however, were unlikely: *1*) child feeding and dietary counseling programs were not being operated by health posts or other facilities in the area during the trial; *2*) all children (12 months and above) with arm circumferences below 11.5 cm, irrespective of xerophthalmia status, were, nonetheless, referred to health posts; *3*) the cutoff for wasting in this study was mild, with 30% of all children having arm circumferences below 13.5 cm, a level that would not have provoked parents with few resources to immediately begin providing their children more food on a regular basis; *4*) high-potency vitamin A is ~90% effective in treating xerophthalmia (West and Sommer 1987), leaving little motivation for parents to sustainably improve the quality of a child's diet once eye signs have disappeared following treatment; and, finally *5*) a recently completed prospective, behavioral study of children with and without a confirmed history of xerophthalmia in the study area has shown that previously xerophthalmic children, 1–2 years after being treated with vitamin A, were less likely to be served food at meals, be washed and given other forms of routine home health care and more likely to be treated harshly than children living in nonxerophthalmic households (Gittelsohn, J., Shankar, A. V., West, K. P., Jr., Faruque, F., Gnawali, T. and Pradahan, E. K., unpublished manuscript). Thus, previously xerophthalmic children are likely to be subjected to poor child caring practices long after xerophthalmia has been successfully treated with vitamin A. These factors would argue against the observed growth effects in xerophthalmic children being due to general improvements in diet or health care at home.

These findings suggest a role for vitamin A in promoting growth in children with clinical signs of vitamin A deficiency, at least in this rural South Asian setting. The effect was especially evident among those who were initially wasted. The lack of effect among children with milder (subclinical) vitamin A deficiency appears to emphasize the role of multiple deficiencies, as well as morbidity, in restricting growth (Allen 1994). It is unlikely that vitamin A supplementation elicits a typical growth response across populations or within the same population over different periods of time. Expectation of growth impact (or lack thereof), in any event, should not drive policies to prevent vitamin A deficiency where reductions in the severity of morbidity and mortality of children can be realized (Beaton et al. 1993, Sommer and West 1996).

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VITAMIN A AND HIV

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Maternal Vitamin A Deficiency and Child Growth Failure during Human Immunodeficiency Virus Infection

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Summary: Although vitamin A is thought to influence growth, the relationship between maternal vitamin A deficiency during pregnancy and child growth is unknown. A longitudinal cohort study of 467 HIV-infected women and their children was conducted in Blantyre, Malawi. The children's weight and height were measured every 3 months until they were 24 months old. Maternal vitamin A deficiency was independently related to both linear and ponderal growth after adjustment for effects of body mass index, child gender, and child HIV status. By 12 months of age, infants born to mothers who were vitamin A–deficient during pregnancy weighed ~8% less ($p < 0.001$) and were ~2% shorter ($p < 0.001$) than infants born to mothers who were not deficient. This study suggests children born to HIV-infected women who are vitamin A–deficient during pregnancy are more likely to have growth failure. **Key Words:** Vitamin A—Retinol—Nutrition—Growth—Human immunodeficiency virus.

Growth failure is one of the most common manifestations of HIV infection in children (1,2). Major factors that may influence the growth of infants born to HIV-infected mothers include maternal nutritional status and infant HIV status (3). There is evidence from large studies that ponderal and linear growth are related to the vitamin A status of preschool children (4). The vitamin A status of the infant is largely dependent on maternal vitamin A available to the fetus in utero and during breastfeeding via the breastmilk. Vitamin A status may play a major role in growth because retinoic acid, the active metabolite of vitamin A, is the gene regulator for growth hormone. (5).

Pregnant women are at a high risk of developing vitamin A deficiency, and in some cultures, nightblindness—the earliest clinical manifestation of vitamin A deficiency—is common enough to be considered a symptom of pregnancy (6). We recently demonstrated that maternal vitamin A deficiency is associated with increased mother-to-child transmission of HIV, low birthweight, and higher infant mortality (7,8). Although maternal vitamin A status is thought to influence child growth, no studies have addressed this issue in humans. The purpose of this study is to determine whether maternal vitamin A deficiency during pregnancy in HIV-infected women is associated with child growth failure.

METHODS

The study population consisted of 567 HIV-infected mothers and their infants who were born at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, from November 1989 to August 1991. The details of this study population have been described elsewhere (7,8). Women presenting to the antenatal clinic of QECH were confirmed to be HIV infected on the basis of a positive results from enzyme-linked immunosorbent assay (ELISA) and

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western blot. Age, weight, and height of the mothers and gestational age based upon the last reported menstrual period were recorded at the first prenatal visit, which occurred during the second or third trimester of pregnancy. Maternal body mass index (Quetelet) was calculated using weight (kg)/height^2 (m) (9). Serum vitamin A levels were measured during the first antenatal visit by high performance liquid chromatography (7). Serum vitamin A levels <1.05 μmol/L were considered consistent with deficiency in adults in this population (8).

At delivery, gestational age was estimated by Ballard score, and the infant's weight was recorded. Mothers and infants were examined every 3 months after delivery, and at each visit the height and weight of the infants were recorded. All infants were weighed on the same calibrated digital scale to the nearest gram. Child length was measured using a child length board. Almost all infants (>99%) in this study population were still at least partially breastfeeding at 12 months of age. At 12 months of age, ELISA and confirmatory western blot were used to determine HIV-1 infection in the infants. All studies were approved by the Malawi Health Sciences Committee (Blantyre) and the Committee on Human Research at Johns Hopkins University (Baltimore).

Generalized nonparametric additive models were used to analyze longitudinal growth data and construct mean growth curves for infants (10,11). Comparisons were made between growth curves of (a) infants born to vitamin A–deficient and nondeficient mothers, (b) infants who were HIV infected and noninfected, and (c) boys and girls. Population means and 95% confidence intervals about the mean were calculated for the two groups from birth to 24 months of age. Generalized estimating equations were used to test for statistical effects of covariates within these correlated longitudinal data.

RESULTS

Of 567 HIV-infected mothers, 421 had infants who survived to 12 months of age, and 146 had infants who died before 12 months of age. Serum vitamin A levels were measured in 474 HIV-infected mothers (in 136 of the 146 mothers whose infants died before 12 months of age and in 338 of the 421 mothers whose infants survived to 12 months of age). Ninety-four women were not included in the vitamin A analysis for the following reasons: 40 mothers had no available serum sample, 40 infants were lost to follow-up between delivery and 12 months, and 14 infants had indeterminate HIV serostatus at age 12 months.

Growth data were available for 328 of the 338 infants of known HIV status who survived to 12 months of age. Among the 328 infants (148 boys and 180 girls) with available growth data, 33 infants died and 40 infants were lost to follow-up between 12 and 24 months of age. The mean linear and ponderal growth curves for boys and girls born to vitamin A–deficient and nondeficient mothers shown in Figs. 1 and 2. For both boys and girls, linear growth and ponderal growth were lower in those born to vitamin A–deficient mothers.

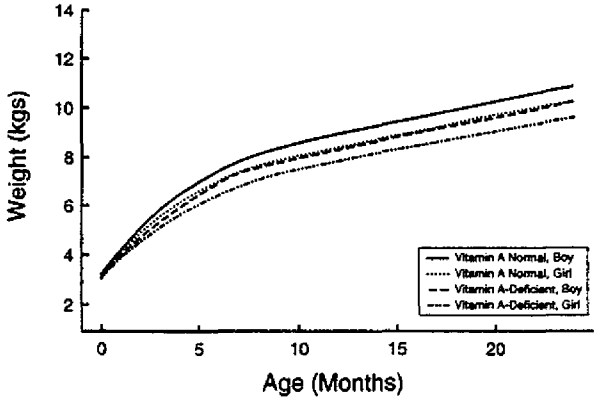

FIG. 1. Weight-for-age of boys and girls born to HIV-infected mothers with and without vitamin A deficiency.

The mean linear and ponderal growth curves for HIV-infected and uninfected children born to vitamin A–deficient and nondeficient mothers are shown in Figs. 3 and 4. As shown in Figs. 3 and 4, HIV-infected children had lower mean linear and ponderal growth than uninfected children, regardless of maternal vitamin A level. Among both HIV-infected and uninfected children, those born to vitamin A–deficient mothers had lower linear and ponderal growth than those born to mothers who were not vitamin A–deficient. For example, by 12 months of age, infants born to vitamin A–deficient mothers weighed ~0.7 kg less (~8% lighter) compared with infants born to mothers who were not deficient in vitamin A. Similarly, at 12 months of age, infants born to vitamin A–deficient mothers were ~1 cm shorter (~1–2% shorter) than infants born to mothers who were not deficient in vitamin A.

Because repeated measures of ponderal and linear

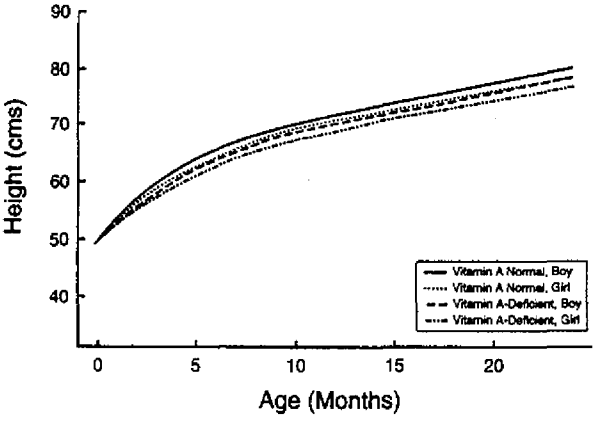

FIG. 2. Height-for-age of boys and girls born to HIV-infected mothers with and without vitamin A deficiency.

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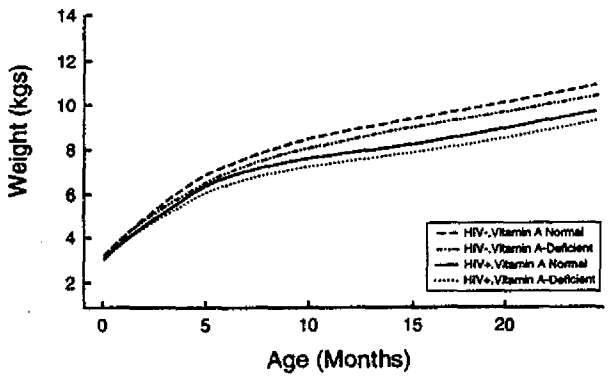

FIG. 3. Measurements of weight-for-age of HIV-infected and uninfected children born to HIV-infected mothers with and without vitamin A deficiency.

growth were obtained from each infant, we used generalized estimation equations that were robust to intraindividual colinearity to estimate simultaneously the effect of maternal vitamin A levels, body mass index, infant gender, infant age, and infant HIV status on growth. In stepwise, bivariate generalized equation models where linear and ponderal growth were the outcome variables, all five variables were significantly related to linear and ponderal growth, respectively, and were fit into final multivariate generalized equation models for growth (Tables 1 and 2).

DISCUSSION

Our study suggests that maternal vitamin A deficiency in HIV-infected mothers is an independent predictor of child growth failure. Children born to HIV-positive women who were vitamin A–deficient

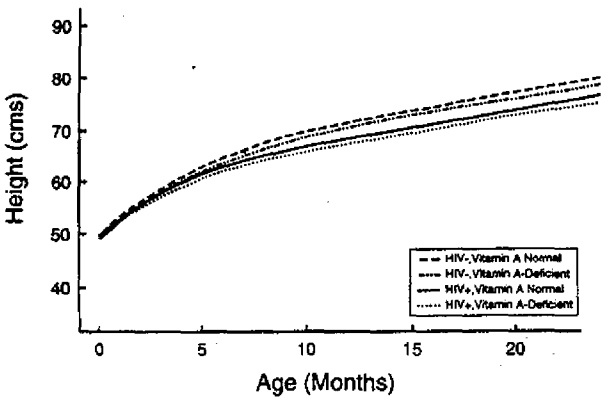

FIG. 4. Measurements of height-for-age of HIV-infected and uninfected children born to HIV-infected mothers with and without vitamin A deficiency.

TABLE 1. *Multivariate generalized equation model for linear growth*

	Estimate (cm)	Standard error	Z-score	p value
Intercept	55.48	0.52	106.87	<0.0001
Age of child	1.15	0.01	94.47	<0.0001
Sex of child	−1.31	0.29	−4.44	<0.0001
Maternal vitamin A	0.83	0.29	2.87	<0.004
Maternal BMI	1.16	0.29	3.98	<0.0001
Infant HIV status	−1.74	0.38	−4.63	<0.0001

For gender, boy = 0, girl = 1; for maternal vitamin A level, deficiency defined as serum vitamin A <1.05 μmol/L; for body mass index (BMI), low and high divided at the median of 23.15 kg/m^2; for infant HIV status, HIV negative = 0, HIV positive = 1.

during pregnancy consistently had lower weight-for-age and height-for-age compared with children born to women who were not vitamin A deficient. To our knowledge, this is the first study in humans to demonstrate that maternal vitamin A deficiency during pregnancy is associated with child growth failure.

The vitamin A status of infants depends on liver stores of vitamin A established through placental transfer while in utero and through the vitamin A in breast milk after delivery (12). Animal studies demonstrate that vitamin A deficiency during pregnancy has a profound effect on growth and mortality of offspring (13). In the human life span, infancy is the most vulnerable period for vitamin A deficiency, and vitamin A deficiency may be overlooked because night blindness is difficult to assess in infants and because Bitot's spots, which are considered pathognomonic for vitamin A deficiency, do not usually occur during infancy.

The impact of maternal vitamin A deficiency on the newborn infant is complex because vitamin A is involved in many different biological functions, including the development of the immune system,

TABLE 2. *Multivariate generalized equation model for ponderal growth*

	Estimate (kg)	Standard error	Z-score	p value
Intercept	4.90	0.16	31.34	<0.0001
Age of child	0.28	0.004	76.94	<0.0001
Sex of child	−0.47	0.093	−4.99	<0.0001
Maternal vitamin A	0.30	0.092	3.30	<0.001
Maternal BMI	1.47	0.092	5.09	<0.0001
Infant HIV status	−0.63	0.106	−5.94	<0.0001

For gender, boy = 0, girl = 1; for maternal vitamin A level, deficiency defined as serum vitamin A <1.05 μmol/L; for body mass index (BMI), low and high divided at the median of 23.15 kg/m^2; for infant HIV status, HIV negative = 0, HIV positive = 1.

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maintenance of mucosal surfaces, and mucosal immunity and growth. The development of the thymus and the normal maturation of T and B cells is dependent on vitamin A (14). Thus, an infant born to a vitamin A–deficient mother may have a greater degree of immune dysfunction than an infant with healthy vitamin A stores. Vitamin A–related immune dysfunction could predispose to more severe infections and associated morbidity, which would be reflected in growth failure. Maternal vitamin A deficiency could also be more directly related to growth failure through regulation of growth hormone (4,5).

This study is limited in that other micronutrient abnormalities in pregnant women that may contribute to child growth failure were not evaluated. Maternal iron deficiency is associated with low birth weight and prematurity (15). Iodine deficiency disorders increase the risk of prematurity, stillbirths, spontaneous abortions, and retarded growth and mental development (16). Zinc deficiency can affect the growth of young children (17). Certain micronutrients may interact; for example, zinc deficiency may affect the availability of circulating vitamin A (18), and vitamin A status can influence the mobilization of hepatic liver stores of iron (19). Further studies are needed to address the effect of multiple micronutrient deficiencies on child growth.

It is unknown whether improving maternal vitamin A status during pregnancy will improve child growth, and the use of vitamin A supplements during pregnancy should be approached with caution because teratogenicity may be a concern with supplemental doses greater than 10,000 IU/day. Periodic vitamin A supplementation of children may increase both linear and ponderal growth, but the observations have not been consistent in all populations (20). Studies are currently in progress in sub-Saharan Africa and in the Indian subcontinent to determine whether maternal vitamin A supplementation will improve child growth and infant mortality.

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Vitamin A and human immunodeficiency virus infection

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By the year 2000 there will be an estimated twenty-six million individuals infected with human immunodeficiency virus (HIV) (Quinn, 1996). About four to five million cumulative cases are expected among children by the year 2000. The areas hardest-hit by the acquired immune deficiency syndrome (AIDS) epidemic are sub-Saharan Africa, south Asia, and south-east Asia, where the prevalence of micronutrient deficiencies is generally higher. More than 90 % of new HIV infections are now occurring in developing countries. Although HIV infection is characterized by a variety of micronutrient deficiencies and wasting (Beach *et al.* 1992; Kotler & Grunfeld, 1995), the relationship between nutritional status and clinical outcome in HIV infection is not well understood. Vitamin A plays a major role in normal immune function (Semba, 1994), and as early as the 1960s it was acknowledged that 'no micronutrient deficiency is more synergistic with infection than that of vitamin A' (Scrimshaw *et al.* 1968). The present review focuses on the relationship between vitamin A, immunity, and HIV infection.

VITAMIN A AND IMMUNITY

Vitamin A deficiency is a nutritionally-acquired immunodeficiency disorder characterized by widespread pathological alterations of mucosal epithelia of the respiratory, gastrointestinal, and genito-urinary tracts, impaired antibody responses to protein antigens, altered cell-mediated immunity, and alterations in T-cell subsets (Semba *et al.* 1992, 1993*b*; Wiedermann *et al.* 1993; Zhao & Ross, 1995; Cantorna *et al.* 1996; Ross, 1996). Vitamin A, through its active metabolite, all-*trans* retinoic acid, regulates genes through nuclear receptors in the steroid and thyroid hormone superfamily (Ross & Ternus, 1993). The genes relevant to immunity which are regulated by vitamin A have not been completely characterized, but probably include those for interleukin-4, interferon-γ (Cantorna *et al.* 1996), interleukin-2 receptor (Sidell *et al.* 1993), and others. Growth and activation of T and B cells are dependent on vitamin A (Buck *et al.* 1990; Garbe *et al.* 1992), and vitamin A supplementation is associated with increases in circulating lymphocytes, including CD4 T-cells and natural killer cells, suggesting that lymphopoiesis is enhanced by vitamin A (Semba *et al* 1993*b*; Ross, 1996).

The potential consequences of the immunodeficiency associated with vitamin A deficiency have been shown in longitudinal studies of preschool children which were conducted in Indonesia in the late 1970s. These studies demonstrated that children with mild vitamin A deficiency were at higher risk of respiratory and diarrhoeal disease and higher mortality (Sommer *et al.* 1983, 1984). A series of community-based clinical trials of vitamin A supplementation for preschool children soon followed in Indonesia, India, Nepal, Sudan, and Ghana, which showed overall that vitamin A supplementation or fortification reduced child mortality by about 20–30 % (Beaton *et al.* 1993). Further analysis showed that vitamin A reduces the severity of diarrhoeal disease but seems to have little effect on respiratory disease (The Vitamin A and Pneumonia Working Group, 1995). High-dose vitamin A supplementation was also shown to reduce morbidity and mortality in

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children with acute, complicated measles (Hussey & Klein, 1990; Coutsoudis *et al.* 1991). These clinical trials demonstrate that vitamin A is an immune enhancer and suggest that vitamin A may have other therapeutic applications as a therapy for HIV infection.

PATHOGENESIS OF VITAMIN A DEFICIENCY

Factors which may contribute to the development of vitamin A deficiency include: low intake of vitamin A-containing foods, anorexia of chronic disease, diarrhoea and malabsorption, concomitant protein–energy malnutrition, liver disease, abnormal urinary losses of vitamin A during infections, the acute-phase response, and accelerated utilization of vitamin A. In many developing countries where HIV infection is common, the intake of vitamin A-containing foods may be marginal. Oesophageal candidiasis, cryptococcal meningitis, and progressive multifocal leukoencephalopathy during HIV infection are associated with anorexia and wasting (Nahlen *et al.* 1993). Diarrhoea and malabsorption syndromes are common during HIV infection and can be caused by a wide variety of pathogens, including cryptosporidiosis and isosporiasis. Fat malabsorption has been reported in HIV-infected individuals and may interfere with vitamin A absorption and metabolism.

Protein–energy malnutrition increases the risk of developing vitamin A deficiency (Smith *et al.* 1973), probably because vitamin A transport relies on a carrier-protein complex of retinol-binding protein and transthyretin. Hepatitis appears to interfere with the metabolism of vitamin A (Smith & Goodman, 1971), and hepatitis B and C are common in many HIV-infected populations. During the acute-phase response, retinol-binding protein may break off from the carrier complex of transthyretin and retinol, allowing abnormal glomerular losses of vitamin A which may reach several times the required daily allowance for vitamin A (Stephensen *et al.* 1994; Alvarez *et al.* 1995). Individuals with HIV infection and AIDS have been shown to have higher abnormal urinary losses of vitamin A compared with controls (Silveira *et al.* 1996). Whether infections accelerate the utilization of vitamin A is not clear; however, the depletion of hepatic vitamin A was shown to be increased in children who incidentally contracted chickenpox during a vitamin A dosing study (Campos *et al.* 1987). It is unknown whether the virulence of HIV itself may influence vitamin A status.

PREVALENCE OF VITAMIN A DEFICIENCY DURING HUMAN IMMUNODEFICIENCY VIRUS INFECTION

The prevalence of vitamin A deficiency may vary widely, depending upon the population. Serum or plasma vitamin A levels have been used as the main indicator of vitamin A status in most of these investigations. In a hospital-based, cross-sectional study of fifty-nine adults with AIDS in New York City who were not taking vitamin supplements, 20 % had serum vitamin A levels $< 0{\cdot}70$ μmol/l (Karter *et al.* 1995). A study of 100 asymptomatic, HIV-infected homosexual men in Miami showed that 18 % had serum vitamin A levels $< 1{\cdot}23$ μmol/l, which were considered to be in the marginal range (Beach *et al.* 1992). In forty-seven HIV-infected adults with wasting syndrome in Portland, Oregon, 14 % had serum vitamin A levels $< 1{\cdot}75$ μmol/l (Coodley *et al.* 1993). A study of seventy-four HIV-infected pregnant women in Thailand showed mean vitamin A levels of 1·23 μmol/l. Direct comparisons of the vitamin A status in these various studies is problematic because of the different cut-offs of serum or plasma vitamin A used to define vitamin A deficiency in adults.

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We investigated the vitamin A status of different HIV-infected populations which included injection drug users, homosexual men, adults in Uganda, pregnant women in Malawi, pregnant women in the USA, and lactating women in Haiti. Comparison of vitamin A levels in these different populations is useful because it may provide clues to the epidemiology and pathogenesis of vitamin A deficiency during HIV infection. Plasma vitamin A levels were measured in 284 HIV-infected injection drug users from a cohort study in Baltimore, Maryland (AIDS Linked to Intravenous Experiences, ALIVE study; Vlahov *et al.* 1991). The frequency distribution of vitamin A levels is shown in Fig. 1. In our studies in adults we have defined vitamin A deficiency as consistent with serum or plasma vitamin A levels $< 1{\cdot}05$ µmol/l, a level at which some individuals may have impairment of biological function (Pilch, 1987). Using this criterion, about 29 % of HIV-infected injection drug users had plasma vitamin A levels consistent with deficiency in adults. Data from the first National Health and Nutrition Examination Survey show that in the normal adult population in the USA, 1–3 % of adults have serum vitamin A levels $< 1{\cdot}05$ µmol/l (Pilch, 1987). In a study of 311 HIV-infected homosexual men (Study to Help the AIDS Research Effort, SHARE) in Baltimore and Washington, DC, the frequency distribution of plasma vitamin A levels suggested that this population was replete in vitamin A (Fig. 2). The frequency distribution is flatter and shifted to the right in this population of homosexual men. Serum vitamin A levels were measured in 522 HIV-infected adults followed in a large cohort study in an outpatient clinic at Old Mulago Hospital, Kampala, Uganda (Fig. 3). The adults in Uganda were HIV seropositive and purified-protein-derivative positive or negative with no radiological evidence of tuberculosis.

Pregnant and lactating women are generally at higher risk of vitamin A deficiency. The frequency distribution of serum vitamin A levels in 474 HIV-infected pregnant women followed in the antenatal clinic at Queen Elizabeth Central Hospital, Blantyre, Malawi is shown in Fig. 4. Of these women 63.3 % had serum vitamin A levels consistent with vitamin A deficiency. In comparison, serum vitamin A levels from 513 HIV-infected pregnant women from the Womens and Infants Transmission Study (WITS), a multicentre study in the USA are shown in Fig. 5 (S. Landesman, D. Burns, D. Cotton, G. Fitzgerald, M. G. Fowler, R. Hershow, J. Pitt, R. D. Semba and C. Zorilla, unpublished results). Of the women in WITS, 22 % had serum vitamin A levels consistent with vitamin A deficiency.

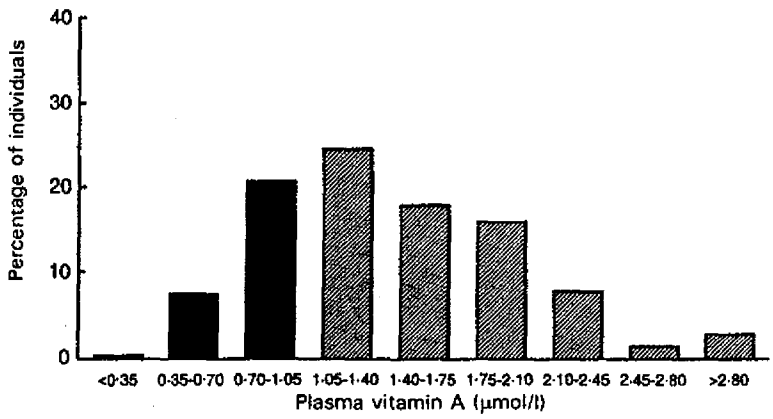

Fig. 1. Frequency distribution of vitamin A levels in 284 HIV-seropositive injection drug users in Baltimore, Maryland. (■), Proportion of individuals with vitamin A levels $< 1{\cdot}05$ µmol/l.

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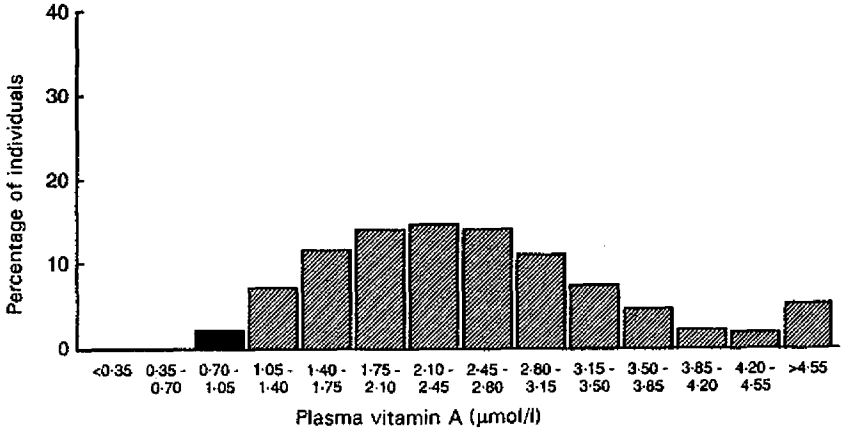

Fig. 2. Frequency distribution of vitamin A levels in 311 HIV-seropositive homosexual men in a cohort from Baltimore, Maryland and the Washington, DC area. (■), Proportion of individuals with vitamin A levels < 1·05 μmol/l.

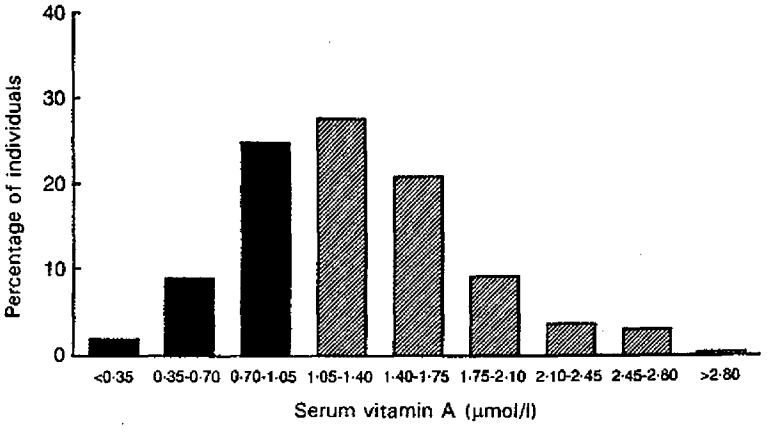

Fig. 3. Frequency distribution of vitamin A levels in 522 HIV-seropositive adults from an outpatient clinic of the Old Mulago Hospital, Kampala, Uganda. (■), Proportion of individuals with vitamin A levels < 1·05 μmol/l.

Overall, the vitamin A status of HIV-infected pregnant women in the USA was better than that in Africa. Vitamin A levels were measured in 126 HIV-infected lactating women at 6 months post-partum who were being followed in an outpatient clinic in Cite Soleil, Port Au Prince, Haiti (J. S. Coberly, R. D. Semba, N. Halsey, J. Desormeaux, D. Jules-Joseph, P. A. Carrenard, M. McBrien, R. Boulos and A. J. Ruff, unpublished results; Fig. 6). Over 75 % of these lactating women had vitamin A levels consistent with vitamin A deficiency.

Comparison of the frequency distributions of vitamin A levels in these six different populations allows some possible generalizations. First, HIV-infected adults from developing countries had lower vitamin A levels than HIV-infected adults in the USA. Such an observation might be expected because the intake of vitamin A-rich or vitamin A-fortified foods is lower in developing countries. Second, HIV-infected injection drug users from inner city Baltimore had lower vitamin A levels than HIV-infected homosexual men

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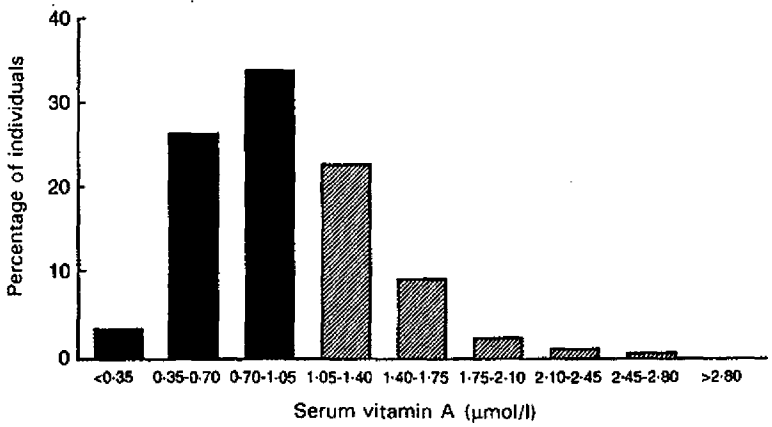

Fig. 4. Frequency distribution of vitamin A levels in 474 HIV-seropositive women seen in the antenatal clinic of the Queen Elizabeth Central Hospital, Blantyre, Malawi. (■), Proportion of individuals with vitamin A levels < 1·05 μmol/l.

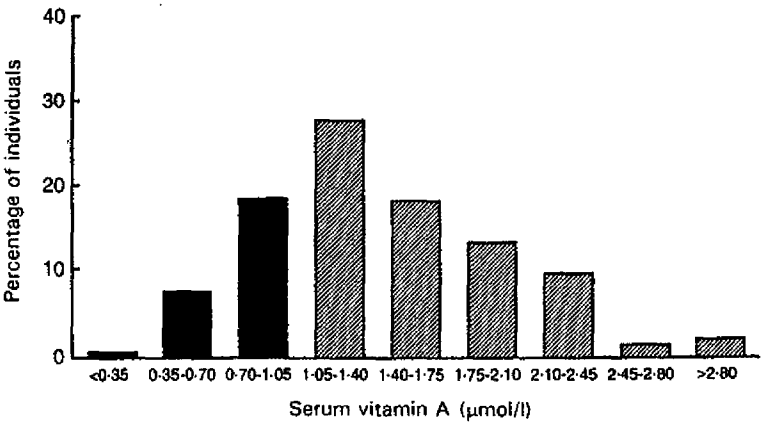

Fig. 5. Frequency distribution of vitamin A levels in 513 HIV-seropositive women in a multicentre cohort study in the USA (Women and Infants Transmission Study). (■), Proportion of individuals with vitamin A levels < 1·05 μmol/l.

from the same region. Dietary and socio-economic factors probably account for these differences. Third, many of the homosexual men were taking vitamin supplements (Tang *et al.* 1993), which suggests that supplementation reduces the risk of having a low vitamin A level, in spite of opportunistic infections associated with HIV infection. Finally, it is well known that pregnancy and lactation are high-risk periods for vitamin A deficiency in women, and these findings show an alarmingly high prevalence of vitamin A deficiency among HIV-infected pregnant and lactating women.

VITAMIN A DEFICIENCY AND MORTALITY

Vitamin A deficiency and HIV infection constitute a combined immunodeficiency syndrome which might be expected to have adverse clinical consequences. Vitamin A

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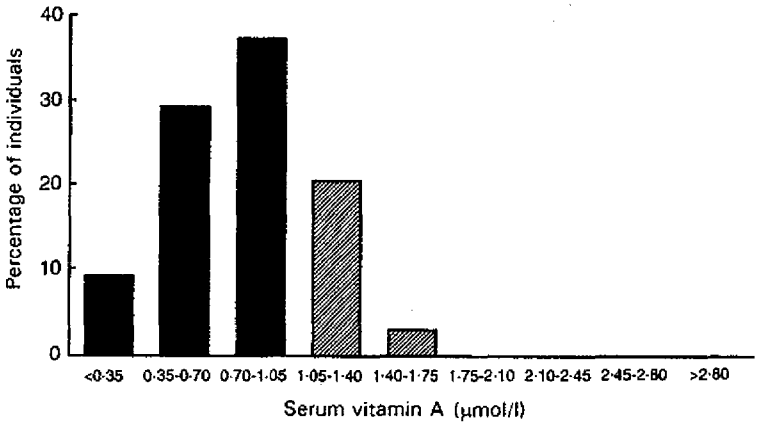

Fig. 6. Frequency distribution of vitamin A levels in 126 HIV-seropositive lactating women from Cite Soleil, Port Au Prince, Haiti. (■), Proportion of individuals with vitamin A levels < 1·05 μmol/l.

deficiency was shown to be associated with increased mortality in a longitudinal study of injection drug users in Baltimore (Semba *et al.* 1993*a*). Adults with vitamin A deficiency had decreased survival by about 1 year. This was corroborated by a larger nested case–control study within the ALIVE study which showed that vitamin A deficiency was an independent risk factor for mortality and was associated with a four-fold increased risk of death (Semba *et al.* 1995*a*). Three other studies from Haiti (Rwangabwoba *et al.* 1996), Uganda, and Miami have recently confirmed the association between vitamin A deficiency and increased mortality during HIV infection, with relative risks for mortality estimated between 2 and 4 in these different studies. In addition, further follow-up in the cohort of HIV-infected women in Malawi shows that women in the lowest quartiles of serum vitamin A levels had significantly higher mortality (Semba, 1997). Although data from five different study populations around the world now demonstrate a similar relationship between vitamin A deficiency and increased mortality in adults, it is unknown whether vitamin A supplementation will reduce mortality in HIV-infected adults.

VITAMIN A DEFICIENCY AND VERTICAL TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS

The period of highest risk for vitamin A deficiency in women is during pregnancy, when there are increased demands for vitamin A because of the growing fetus. Epidemics of nightblindness, the earliest clinical manifestation of vitamin A deficiency, were common among pregnant women in Europe in the nineteenth century and early part of this century before dietary improvements (Birnbacher, 1928). Nightblindness was so common in pregnant women near Bombay, India, that it was considered to be a normal symptom of pregnancy, along with morning sickness (Dixit, 1966). It has been shown recently in a case–control study in Nepal that pregnant women with nightblindness have significantly higher infectious disease morbidity than matched controls without nightblindness (Christian *et al.* 1996). As shown in Figs. 4 and 6, vitamin A deficiency has the highest prevalence among pregnant and lactating women. The major factor contributing to the high prevalence of nightblindness during pregnancy is probably insufficient intake of vitamin A-rich foods.

The rates of vertical transmission (mother-to-child transmission) of HIV generally are estimated to be 10–40 %, with rates being higher in developing countries. Prolonged duration of breast-feeding in developing countries was generally thought to account for the higher rates in areas such as sub-Saharan Africa, because HIV may be present in breast milk. Other known risk factors for vertical transmission include prolonged duration of rupture membranes at delivery (Landesman *et al.* 1996), high maternal HIV viral load (Dickover *et al.* 1996), low maternal CD4 count, and chorioamnionitis (Temmerman *et al.* 1995).

Given the potential effects of vitamin A deficiency on immunity, and the high prevalence of vitamin A deficiency during pregnancy, we investigated the relationship between vitamin A deficiency and vertical transmission. HIV-infected pregnant women in Malawi who were vitamin A-deficient were three to four times more likely to transmit HIV to their infants (Semba *et al.* 1994). There was a strong association between low maternal vitamin A levels and increased vertical transmission after controlling for CD4 count, maternal age, and maternal BMI. Infant mortality was higher among those born to vitamin A-deficient HIV-infected pregnant women, reaching 93 % mortality among infants born to the most-severely-vitamin A-deficient women (Semba *et al.* 1995*b*). Further studies in Malawi (Semba, unpublished results) have recently shown that HIV-seronegative pregnant women with serum vitamin A levels consistent with deficiency also have significantly higher infant mortality. Thus, the findings of maternal vitamin A deficiency and increased infant mortality are consistent among both HIV-seropositive and -seronegative women.

Whether low serum vitamin A levels are a 'marker' for increased vertical transmission or are causally involved is unknown. In experimental animal models, vitamin A deficiency during pregnancy is associated with chorioamnionitis (Noback & Takahashi, 1978), a placental infection which is a known risk factor for vertical transmission. In addition, animals made deficient in vitamin A during pregnancy have low birth weight and high mortality of offspring, which is consistent with the findings from human studies. Vitamin A deficiency may have serious consequences for immune function in the mother as well as immune development in the fetus, but these biological mechanisms have not been characterized. The association between maternal vitamin A deficiency and vertical transmission has been confirmed in Rwanda (Semba, unpublished results), Haiti (J. S. Coberly, R. D. Semba, N. Halsey, J. Desormeaux, D. Jules-Joseph, P. P. Carrenard, M. McBrien, R. Boulos and A. J. Ruff, unpublished results), and in Baltimore and New York City (Greenberg *et al.* 1996). A recent study from Kenya shows that HIV-infected women with vitamin A deficiency have a greatly increased risk of HIV load in breast milk, which suggests a possible mechanism for increased transmission through breast-feeding (Nduati *et al.* 1995).

VITAMIN A DEFICIENCY AND VIRAL LOAD

High circulating HIV viral load has been shown to be a risk factor for HIV progression (Ferre *et al.* 1995) and vertical transmission (Dickover *et al.* 1996). The relationship between serum vitamin A levels and viral load is of potential importance, because the HIV genome contains a retinoic acid response element in the long terminal repeat (Orchard *et al.* 1993), and all-*trans* retinoic acid, the active metabolite of vitamin A, has been shown to influence HIV replication (Poli *et al.* 1992; Turpin *et al.* 1992). In a cross-sectional study of 284 HIV-infected injection drug users from the ALIVE cohort, no relationship was found between plasma vitamin A levels and viral load (Semba, unpublished results). In a study of homosexual men from the Multicentre AIDS Cohort Study, there was no relationship

between serum vitamin A levels and viral load (Semba, unpublished results). A randomized, double-masked, placebo-controlled clinical trial of high-dose vitamin A supplementation for 120 HIV-infected adults shows that vitamin A supplementation has no effect on viral load (Semba, unpublished results). In a related study, a phase I/II trial of all-*trans* retinoic acid treatment for Kaposi's sarcoma showed no effect of retinoic acid on HIV p-24 antigenaemia, suggesting that retinoic acid had no effect on viral load (Bailey *et al.* 1995).

These studies suggest that vitamin A levels and viral load are independent risk factors during HIV infection, and that vitamin A deficiency is not a marker for viral load. High-dose vitamin A supplementation does not seem to have an effect on viral load levels, and this finding has implications for clinical trials of antenatal supplementation of vitamin A as a strategy to reduce mother-to-child transmission of HIV. These findings refute the idea that vitamin A supplementation would work in the same manner as an anti-retroviral medication, although some clinical trials are designed as if this were an expected biological effect. Perhaps it is more important that these findings show that vitamin A supplementation does not seem to up-regulate the expression of HIV, a theoretical consideration raised by *in vitro* studies. Should clinical trials of vitamin A therapy to reduce mother-to-child transmission show a beneficial clinical effect, these studies suggest that the biological mechanism(s) would probably be mediated through some other means, such as reduction in chorioamnionitis or improvement of immune development in mother and infant.

VITAMIN A AS THERAPY IN HUMAN IMMUNODEFICIENCY VIRUS INFECTION

The community- and hospital-based clinical trials of vitamin A for child survival and treatment of acute measles provide compelling rationale for investigating the use of vitamin A as therapy in HIV infection. Epidemiological studies demonstrate that vitamin A deficiency is common in HIV-infected pregnant women, injection drug users in the inner city, and in adult populations in developing countries. In sub-Saharan Africa, where anti-retroviral and prophylactic therapies may be prohibitively expensive, the optimization of micronutrient status may be among the affordable strategies available for potentially prolonging survival and reducing vertical transmission of HIV. Two recent clinical trials of vitamin A therapy for HIV-infected children suggest that vitamin A supplementation reduces diarrhoeal morbidity (Coutsoudis *et al.* 1995) and improves immunity (Hussey *et al.* 1996). Four clinical trials are currently in progress in Tanzania, South Africa, Malawi, and Zimbabwe to determine whether antenatal vitamin A supplementation can reduce vertical transmission and infant mortality. A large clinical trial in Uganda is addressing the question of whether periodic vitamin A supplementation can improve survival for HIV-infected children. A recent World Bank (1993) analysis has shown that vitamin A supplementation has the second-highest cost-benefit effect of forty-seven major health interventions studied. It remains to be determined whether vitamin A supplementation will have therapeutic benefit in reducing vertical transmission of HIV, infant mortality, and mortality of young children.

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Overview of the potential role of vitamin A in mother-to-child transmission of HIV-1

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Vitamin A is an essential micronutrient for normal immune function. Vitamin A deficiency is common among human immunodeficiency virus (HIV)-infected pregnant women and is associated with higher mother-to-child transmission of HIV-1 and increased infant mortality. The biological mechanisms by which vitamin A deficiency could influence mother-to-child transmission of HIV-1 include impairment of immune responses in both mother and infant, abnormal placental and vaginal pathology and increased HIV viral burden in breastmilk and blood. Clinical trials are currently in progress to determine whether daily micronutrient supplementation, including vitamin A, during pregnancy can reduce mother-to-child transmission of HIV-1. ☐ *Acquired immunodeficiency syndrome, HIV-1, vitamin A, vertical transmission*

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Vitamin A, or all-*trans*-retinol, is an essential micronutrient for immunity, growth, reproduction, maintenance of epithelial surfaces and vision (1). This fat-soluble vitamin is found either as preformed vitamin A in liver, cod-liver oil, butter, egg yolks and other dairy products, or as provitamin A carotenoids, such as β-carotene, in carrots, dark-green leafy vegetables and various orange and yellow fruits and vegetables. The liver contains approximately 90% of the body's stores of vitamin A, and the liver has the capacity to store many months' worth of vitamin A, with a longer storage capacity for adults than children. Vitamin A is released into the blood in a one-to-one molar complex with transthyretin and retinol-binding protein, and it is utilized by all nucleated cells in the body. In the cytoplasm, all-*trans*-retinol is converted to all-*trans*-retinoic acid, the main active metabolite of vitamin A. The cell nucleus contains receptors that belong to the superfamily of steroid and thyroid hormone receptors (2) and bind to both retinoic acid and to specific DNA sequences. Through these receptors, known as retinoic acid receptors (RAR) and retinoid-X receptors (RXR), the active metabolites of vitamin A effect gene transcription (3). It is thought that vitamin A may ultimately be involved in the regulation of more than 300 genes. The recent discovery of these nuclear receptors in the last decade has lent credence to the concept that vitamin A acts as a potent gene regulator.

Vitamin A deficiency as an immunodeficiency disorder

Vitamin A deficiency can occur due to such factors as inadequate intake of vitamin A-containing foodstuffs, malabsorption of vitamin A, impaired storage due to liver disease and accelerated utilization and urinary loss of vitamin A during acute and chronic infection. Vitamin A deficiency has been traditionally defined on the basis of ophthalmological signs and symptoms of deficiency, i.e. nightblindness and xerophthalmia, or other biochemical indicators of low circulating vitamin A levels or stores. Children or adults with vitamin A deficiency are more susceptible to infectious diseases, and longitudinal studies have suggested that vitamin A-deficient children may have a fourfold increased risk of death in developing countries (4).

Vitamin A deficiency itself is a mild immunosuppressive disorder that is perhaps the most common of what has recently been dubbed the "Nutritionally Acquired Immunosuppressive Disorders," or NAIDS (5). The main immune abnormalities caused by a deficiency in vitamin A include impaired antibody responses to T-cell-dependent protein antigens, impaired cell-mediated immunity, atrophy of lymphoid tissues such as thymus, spleen, lymph nodes and Peyer's patches, altered circulating T-cell subsets and metaplasia of ocular, respiratory, gastrointestinal, and genito-urinary mucosal surfaces. There is a wealth of animal and human studies that demonstrate these abnormalities (1). Vitamin A deficiency is known to increase the risk of subsequent infections, and infections increase the subsequent risk of vitamin A deficiency, leading to a vicious cycle of infection and deficiency, sometimes ending in death.

Vitamin A deficiency during HIV infection

Given the close relationship between vitamin A deficiency, compromised immunity and infections, we began detailed

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longitudinal observations to determine whether vitamin A deficiency may influence the clinical course of human immunodeficiency virus (HIV) infection. At that time there were cross-sectional studies suggesting that perhaps 10–30% of HIV-infected adults might be deficient in vitamin A (6–8). We commenced a longitudinal study of vitamin A deficiency in HIV-infected intravenous drug users (IDUs) in Baltimore, Maryland (9). Plasma vitamin A levels were measured in a random sample of 179 individuals from a cohort of over 2000 IDUs who were participating in the AIDS Linked to Intravenous Experiences, or ALIVE Study (10). This study suggested that about 15% of IDUs had plasma vitamin A levels consistent with deficiency, and those who were vitamin A deficient had a fourfold increased risk of death and shortened survival compared to those without vitamin A deficiency. Because of the limited size of this study, we wanted to confirm these findings with a larger, more rigorous epidemiological study design. We conducted a nested case-control study comparing those who died with AIDS and infections with those who survived in the similar time period, matched by CD4 T-cell count on enrollment in the ALIVE cohort (11). We matched 50 cases with 235 controls. The nested case-control study suggested that vitamin A deficiency, wasting and low CD4 T-cell count were all independently predictive of death during HIV infection.

To summarize, a variety of cross-sectional studies indicate that vitamin A deficiency is a common micronutrient deficiency during HIV infection (6–8, 12), and longitudinal studies suggest that serum vitamin A levels consistent with vitamin A deficiency are associated with increased risk of death (9, 11). In the absence of clinical trial data, one cannot definitively conclude that vitamin A deficiency leads to higher mortality during HIV infection. However, among children, supplementation with vitamin A during HIV infection may potentially reduce morbidity (13), and there have been over a dozen clinical trials showing that vitamin A supplementation can reduce morbidity and mortality in general among non-HIV-infected individuals (1).

Vitamin A deficiency and mother-to-child transmission of HIV-1

The mother-to-child transmission rate of HIV-1 is generally higher in developing countries than in developed countries. For example, in Malawi the rate is about 34%, compared with 13% in Europe (14, 15). Although the difference between these rates was usually attributed to longer duration of breastfeeding in developing countries, it seemed reasonable that nutritional abnormalities might also contribute to the higher mother-to-child transmission rates in developing countries.

Nightblindness, the mildest clinical manifestation of vitamin A deficiency, is common during pregnancy in many developing countries, and in some places it may be so common that it is considered to be a normal part of pregnancy, along with morning sickness (16). Epidemics of nightblindness among pregnant women were frequent in Europe until dietary improvements occurred after the 1930s (17). Other common micronutrient deficiencies during pregnancy include iron and folate deficiency. Given the high prevalence of vitamin A deficiency during pregnancy and studies that suggested a relationship between vitamin A deficiency and mortality during HIV infection, it seemed reasonable to commence an investigation to determine if maternal vitamin A status is related to vertical transmission rates, child morbidity and mortality and maternal mortality. We studied the relationship between vitamin A deficiency and clinical outcome within the context of a large natural history study of HIV infection in pregnant women in Blantyre, Malawi, which was supported by the National Institutes of Health as one of the International Collaboration in AIDS Research (ICAR) projects.

The study population consisted of 567 HIV-infected mothers and their infants who were born at Queen Elizabeth Central Hospital in Blantyre, Malawi, from November 1989 to August 1991. Serum vitamin A levels were measured during the second or third trimester of pregnancy, and mothers and infants were followed from delivery until the infants reached 1 year of age. The relationship between maternal vitamin A levels and vertical transmission of HIV-1 was studied in 338 HIV-positive mothers whose infants survived to 12 months of age, at which time the HIV serostatus was determined by ELISA. Mean vitamin A levels in 74 mothers who transmitted HIV to their infants was $0.86 \pm 0.03\,\mu mol\,l^{-1}$ compared with $1.07 \pm 0.02\,\mu mol\,l^{-1}$ in 264 mothers who did not transmit HIV to their infants ($p < 0.0001$) (18). Both maternal serum vitamin A levels and maternal percentage CD4 levels were independently related to mother-to-child transmission of HIV. This study suggested that maternal vitamin A deficiency might contribute to increased mother-to-child transmission of HIV-1.

Maternal vitamin A deficiency and mortality of infant and mother

We then studied the relationship between maternal vitamin A levels and infant mortality among women in the same cohort in Malawi (19). This included the 338 HIV-positive mothers whose infants survived to 12 months of age and 136 mothers whose infants died before 12 months of age. Infant mortality rates were the highest among infants born to the most vitamin A-deficient mothers, with 93.3% mortality among infants born to mothers with vitamin A levels $< 0.35\,\mu mol\,l^{-1}$, compared with 14.2% infant mortality among the most vitamin A-replete mothers (serum vitamin A $> 1.75\,\mu mol\,l^{-1}$). In a multivariate analysis, both maternal serum vitamin A levels and maternal age were significantly related to infant mortality. Infant birthweight was also significantly lower among

those born to vitamin A-deficient mothers, although the magnitude of the difference was relatively small (19). Although animal models have long suggested a relationship between vitamin A deficiency during pregnancy and adverse outcomes such as low birthweight and higher mortality of offspring (20), it was previously unknown whether this relationship existed in humans. We also examined the relationship between maternal vitamin A levels and maternal mortality during further subsequent follow-up in Malawi. In follow-up until the end of 1993, 38 of 474 HIV-infected mothers died. We divided the serum vitamin A levels into quartiles, and those mothers in the lowest quartile of vitamin A levels during pregnancy had significantly lower survival than the higher quartiles of serum vitamin A (Fig. 1). This study confirms previous studies showing that low vitamin A levels are associated with increased risk of mortality (9, 11).

Potential biological mechanisms—some hypotheses

Because of the essential role that vitamin A plays in both antigen-specific immunity and non-specific immunity, i.e. maintenance of mucosal barriers, it seems reasonable that vitamin A deficiency could influence mother-to-child transmission of HIV, infant mortality and maternal mortality. Although the specific biological mechanism(s) is unknown, there are several hypotheses that could be examined:

(i) Hypothesis 1: *Maternal vitamin A deficiency impairs antibody responses to HIV, thus conferring a lower titer of passively acquired antibodies to HIV to the infant, resulting in less passive protection and higher vertical transmission of HIV.* The hallmark of vitamin A deficiency is the impairment of antibody responses to T-cell-dependent antigens (21). The exact role of "neutralizing" antibodies to HIV is unclear and may depend upon stage of HIV infection and other factors. We attempted to gain some insight into the relationship between maternal vitamin A levels and antibody responses to HIV antigens by examining the presence or absence of bands to different HIV antigens on the Western blot of pregnant mothers in the Malawi study. Mothers with a positive HIV ELISA (Wellcozyme, Wellcome Diagnostics, Dartford, UK) had their HIV status confirmed by Western blot (Bio-Rad Laboratories, Hemel Hempstead, UK). The Western blot consisted of bands to *env* (gp41, gp120, gp160), *gag* (gp17, gp24) and *pol* (p31, p51, p55, p66) antigens. Among 470 HIV-infected mothers whose Western blot bands were graded, vitamin A deficiency (serum levels $< 1.05\ \mu mol\ l^{-1}$) was associated with a significantly higher proportion of individuals missing bands to gp41, gp120, gp17, p31 and p51 (Fig. 2). Although the method of assessing antibodies to different HIV antigens was semi-quantitative, these data suggest that maternal vitamin A deficiency is associated with decreased antibody responses to 5/9 HIV antigens in the Western blot assay. Further studies using more precise quantitative techniques are necessary to determine whether antibody titer to HIV is altered in HIV-infected mothers with vitamin A deficiency.

(ii) Hypothesis 2: *Maternal vitamin A deficiency causes lowered antibody responses to a wide variety of pathogens, thus conferring lower titers of passively acquired antibodies to a variety of pathogens to the infant, resulting in higher infant morbidity and mortality.* This is currently a major gap in knowledge, as little is known for either HIV-infected or non-infective mothers and their infants. For example, it is unknown whether maternal vitamin A

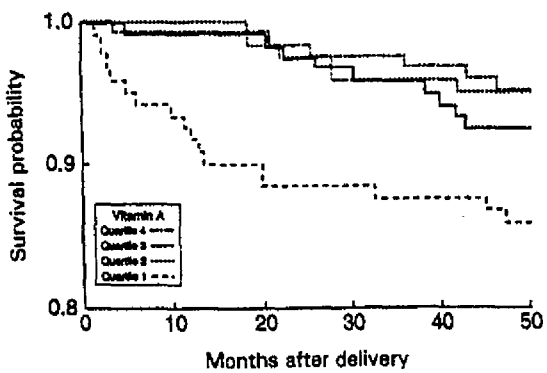

Fig. 1. Survival rates of HIV-infected mothers as related to quartile of vitamin levels, with quartile 1 as the lowest and quartile 4 as the highest.

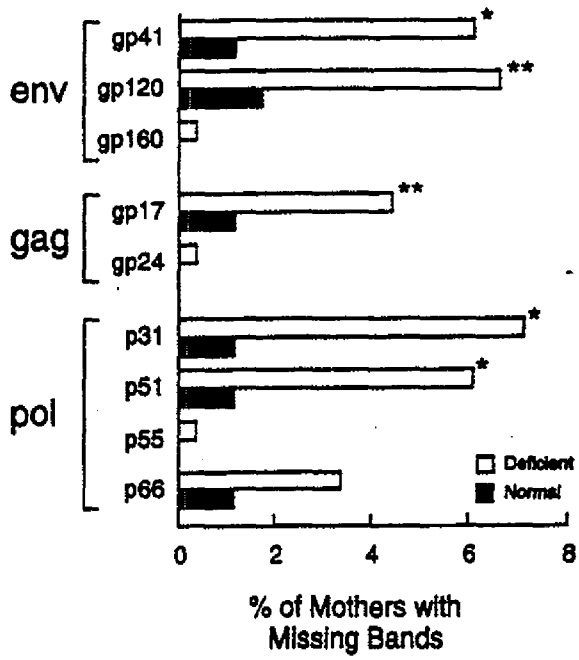

Fig. 2. Percentage of HIV-infected mothers with missing bands on Western blot for HIV-1, as related to vitamin A deficiency: $*p < 0.001$ and $**p < 0.05$.

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deficiency would result in lower titers of passively transferred antibodies against measles to the infant.

(iii) Hypothesis 3: *Maternal vitamin A deficiency causes abnormal placental pathology, i.e. chorioamnionitis, allowing higher vertical transmission of HIV.* Animal studies suggest that deficiency of vitamin A is associated with increased placental infections, low birthweight and prematurity (22, 23). Currently studies are in progress to determine whether maternal vitamin A deficiency or other micronutrient deficiencies are associated with increased placental pathology in two large HIV-infected cohorts in Uganda and Thailand.

(iv) Hypothesis 4: *Maternal vitamin A deficiency is associated with increased HIV viral burden in breastmilk, allowing higher vertical transmission of HIV through breastfeeding.* A recent study in Kenya indicates that HIV-infected lactating women with vitamin A deficiency and CD4 T-cell count < 400 cells μl^{-1} have more than a 20-fold higher risk of having high levels of HIV-1 DNA in breastmilk compared to women who are non-deficient (24). This relationship has recently been confirmed in a separate study in Haiti (Coberly et al., submitted for publication). Whether vitamin A supplementation, either during pregnancy or after delivery, will reduce HIV viral burden in breastmilk and HIV transmission through breastfeeding is unknown and currently being addressed by clinical trials in Malawi, Tanzania and South Africa.

(v) Hypothesis 5: *Maternal vitamin A deficiency is associated with increased HIV viral burden.* Vitamin A-associated immune impairment may allow higher HIV viral burden, which itself may cause higher vertical transmission of HIV. We have examined HIV viral load and plasma vitamin A levels in IDUs and have not found a relationship between vitamin A levels and HIV viral load (Semba et al., submitted for publication).

(vi) Hypothesis 6: *Maternal vitamin A deficiency causes abnormalities of the vaginal mucosa, resulting in more bleeding at the time of delivery and more exposure of the infant to HIV-infected blood.* There is a paucity of data from humans, but animal studies show that during vitamin A deficiency there is loss of mucus and squamous metaplasia in the vagina (25). Whether vitamin A deficiency and pathological changes in the vagina might increase susceptibility to bleeding, infection or increased HIV in the birth canal is unknown.

(vii) Hypothesis 7: *Vitamin A deficiency during pregnancy in the mother also causes deficiency in the fetus, allowing vitamin A-related immune impairment in the infant and increased vertical transmission of HIV and higher infant mortality.* Mothers who are deficient in vitamin A during pregnancy are more likely to have infants who are also deficient (26), and this relationship appears to occur during HIV infection (Coberly et al., submitted for publication). A relationship between maternal vitamin A deficiency and infant immune dysfunction has not yet been demonstrated.

Further unanswered questions

The major micronutrient deficiencies that are common during pregnancy are those of vitamin A, folate and iron. Other micronutrient deficiencies are less common or not known to be associated with adverse birth outcome, thus vitamin A deficiency was a reasonable point at which to begin investigations. Are other micronutrient deficiencies involved in increased vertical transmission of HIV and increased infant mortality? One of the difficulties in studying this question retrospectively from cohorts of HIV-infected women is that the archived samples were often not originally handled in consideration of subsequent micronutrient studies. For example, serum samples for zinc need to be drawn and stored in special trace element vials. Further prospective studies are needed to investigate this issue.

Are low vitamin A levels only a "marker" for severity of infection rather than true vitamin A deficiency? This question was also raised with other diseases, such as acute measles, until it was demonstrated that vitamin A supplementation could reduce measles mortality by greater than 50% (27). During HIV infection, vitamin A levels consistent with deficiency are independently associated with adverse clinical outcome during HIV infection, controlling for levels of CD4 T cells, protein energy status and other factors. Vitamin A levels are not correlated with circulating HIV viral burden (Semba et al., submitted for publication). No studies to date have shown that vitamin A is merely a "marker" for a more important factor, and this reductionist argument may be settled by clinical trials. Is there a similar relationship between maternal vitamin A deficiency and mother-to-child transmission of HIV-1 in developed countries? A preliminary study suggests that such a relationship may exist among inner city women in New York and Baltimore (Greenberg, Semba et al., unpublished data). Could vitamin A supplementation actually increase HIV viral burden and worsen the clinical course of HIV infection? *In vitro* studies show that, depending on cell line and culture conditions, retinoids can either up- or down-regulate HIV replication (28, 29). The HIV viral burden is currently being measured in a clinical trial of vitamin A supplementation involving 120 HIV-positive IDUs (Semba et al., unpublished data).

Clinical trials of vitamin A for reducing vertical transmission of HIV

Although the temporal relationship between vitamin A deficiency during pregnancy and increased vertical transmission of HIV-1 and infant mortality suggest that vitamin A is playing an important role, the ultimate proof of a causal role will be determined by clinical trials of vitamin A supplementation during pregnancy in HIV-infected women. Four clinical trials are currently being conducted in sub-Saharan Africa in Malawi, Tanzania, South Africa and Zimbabwe. A comparison of these clinical trial designs

Table 1. Clinical trials of vitamin A and vertical transmission of HIV-1.

Site	*n*	Treatment groups	Outcomes	Collaborators
Blantyre, Malawi	700	(1) Vitamin A, iron, folate (2) Iron, folate	(1) Vertical transmission (2) Infant morbidity and mortality (3) HIV in breast milk	Johns Hopkins University University of Malawi
Durban, South Africa	750	(1) Vitamin A, β-carotene, iron, folate (2) Iron, folate	(1) Vertical transmission (2) HIV in breast milk	University of Natal Columbia University
Dar Es Salaam Tanzania	960	(1) Vitamin A, β-carotene (2) Vitamin A, β-carotene, multivitamins (3) Multivitamins only (4) Placebo only	(1) Vertical transmission, (2) Progression to AIDS in mothers	Harvard University
Harare, Zimbabwe	600	(1) Multivitamins, iron, folate (2) Placebo only	(1) Vertical transmission	Royal Veterinary and Agricultural University, Copenhagen University of Zimbabwe, Blair Research Laboratory

is shown in Table 1. Two of the four studies include iron and folate for all women participants, while the studies in Tanzania and Zimbabwe have a true placebo arm, i.e. women receiving no nutritional supplementation. Only the Malawi study is a clinical trial examining vitamin A supplementation, while the others are examining a combination of vitamin A and β-carotene, or in the case of Zimbabwe, the use of multiple micronutrient supplements. The designs of these vitamin A clinical trials raise further questions regarding the dose of vitamin A, whether to incorporate β-carotene as well, the duration of supplementation, the addition of other micronutrients and combination with other therapies. These clinical trials should provide information on the effects of vitamin A on both early transmission (during pregnancy and delivery) and late transmission (through breastfeeding) of HIV. Results of these clinical trials regarding the impact of vitamin A on vertical transmission of HIV are expected later this year.

Ideally, an intervention to stop transmission of HIV from mother to child would have the following attributes: (i) inexpensive; (ii) easy to administer; (iii) not requiring HIV testing of pregnant women, i.e. given to all pregnant women as routine antenatal care; (iv) safe with no side effects; and (v) not requiring monitoring. If the clinical trials mentioned above should demonstrate that vitamin A supplementation has efficacy in reducing mother-to-child transmission of HIV, it might meet the attributes for an ideal intervention.

Summary and conclusions

In many parts of the world, the AIDS pandemic is predicted to reverse much progress in the last 50 years with regard to morbidity and mortality of infants, children and adults. Many factors, such as stage of disease in the mother, prolonged breastfeeding and birth order, have been shown to increase the risk of vertical transmission of HIV. Until recently, the nutritional status of HIV-infected pregnant women has been ignored. Vitamin A deficiency is common in HIV-infected pregnant women and is associated with increased vertical transmission of HIV, higher infant mortality, low birthweight, impaired antibody responses to HIV and increased maternal mortality. Studies suggest that possible biological mechanisms include increased HIV viral burden in breastmilk and impaired antibody responses to HIV in vitamin A-deficient mothers. Clinical trials are currently in progress in sub-Saharan Africa to determine if simple supplementation with vitamin A or other micronutrients will reduce mother-to-child transmission of HIV.

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Association between serum vitamin A and E levels and HIV-1 disease progression

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Objective: To examine the associations between serum vitamin A and E levels and risk of progression to three key outcomes in HIV-1 infection: first AIDS diagnosis, CD4+ cell decline to < 200 cells × 10^6/l, and mortality.

Design: Non-concurrent prospective study.

Methods: Serum levels of vitamins A and E were measured at the enrollment visit of 311 HIV-seroprevalent homo-/bisexual men participating in the Baltimore/Washington DC site of the Multicenter AIDS Cohort Study. Cox proportional hazards models were used to estimate the relative hazard of progression to each outcome over the subsequent 9 years, adjusting for several independent covariates.

Results: Men in the highest quartile of serum vitamin E levels (≥ 23.5 μmol/l) showed a 34% decrease in risk of progression to AIDS compared with those in the lowest quartile [relative hazard (RH), 0.66; 95% confidence interval (CI), 0.41–1.06)]. This effect was statistically significant when comparing the highest quartile of serum vitamin E to the remainder of the cohort (RH, 0.67; 95% CI, 0.45–0.98). Associations between serum vitamin A levels and risk of progression to AIDS were less clear, but vitamin A levels were uniformly in the normal to high range (median = 2.44 μmol/l). Similar trends were observed for each vitamin with mortality as the outcome, but neither vitamin was associated with CD4+ cell decline to < 200 cells × 10^6/l. Men who reported current use of multivitamin or single vitamin E supplements had significantly higher serum tocopherol levels than those who were not taking supplements ($P = 0.0001$). Serum retinol levels were unrelated to intake of multivitamin or single vitamin A supplements.

Conclusions: These data suggest that high serum levels of vitamin E may be associated with slower HIV-1 disease progression, but no relationship was observed between retinol levels and disease progression in this vitamin A-replete population.

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Keywords: AIDS, diet, HIV-1 infection, nutrition, retinol, tocopherol, vitamin A, vitamin E

Introduction

The time to development of AIDS in individuals infected with HIV-1 varies considerably between individuals. The median incubation period for AIDS has been estimated at approximately 10 years [1]. However, the range is large, with some patients progressing to AIDS within 2 years of infection whereas others may remain AIDS-free for 15 years or more [1,2]. Both viral factors and the host immune response to HIV-1 appear to be important in influencing the length of time between infection and the onset of AIDS [3]. HIV-1

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infected individuals with non-progressive disease (those who remain healthy with stable CD4+ cell counts) are thought to be infected with less virulent HIV strains or may have the ability to mount a highly effective immune response [4].

Nutritional status has been hypothesized to be a potential cofactor which could modify the course of HIV-1 disease progression through its effects on immune function [5,6]. Vitamins A and E are both important for optimal functioning of the immune system [7]. Deficiencies of these vitamins have been found to impair immune responses in a variety of studies [7,8]. There is ample evidence from both human and animal studies that supplementation with vitamins A and/or E enhances immunity and resistance to various infectious diseases [7–13]. Previous studies have found significant proportions of HIV-1-seropositive individuals with low serum levels of either or both vitamins [14–18]. Low serum levels or low intakes of vitamin A in HIV-1 infection have been associated with lower CD4+ cell counts [17], increased maternal-fetal transmission [18], increased mortality from AIDS or infections [19], and increased risk of progression to AIDS [20]. Low serum vitamin E levels in HIV-1-infected individuals have been correlated with a higher degree of lipid peroxidation [21], increased p24 antigenemia [22], decreased plasma levels of polyunsaturated fatty acids [22], and increased plasma immunoglobulin (Ig) E levels [23]. Supplementation with vitamins A and E has been shown to improve immune parameters in several murine AIDS models [10,24–26]. There is also evidence that vitamin A supplementation in children with HIV-1 may decrease diarrhoeal symptoms [27].

In this study we examine the association between serum levels of vitamins A and E and subsequent disease progression in a cohort of HIV-1 infected homo-/bisexual men.

Methods

Eligible subjects were 341 men who were HIV-1 seropositive upon enrollment into the Baltimore/Washington, DC site of the Multicenter AIDS Cohort Study (MACS), a prospective study on the natural history of HIV-1 infection in homosexual and bisexual men. The objectives, design and recruitment protocol of the MACS have been described elsewhere [28]. Of those who were eligible, 312 (91%) had serum available in the repository from their 1984 baseline study visit. The remaining HIV-1-seropositive individuals (n = 29) did not differ significantly with respect to age, race, education, cigarette smoking, CD4+ cell counts or weight (data not shown). Concentrations of vitamins A and E were estimated for 311 of the 312 subjects. Levels were determined using high performance liquid chromatography procedures [29]. The stability of vitamins A and E in sera stored for long periods of time has been examined in several studies and results have indicated that under optimal storage conditions, both vitamins can be quite stable for periods of several years and through several freeze–thaw cycles [30–33]. Serum albumin and C-reactive protein (CRP) levels were assessed in 308 of the subjects (by Dr Ranjit Chandra, St John's University, St John's, Newfoundland, Canada, 1993). Serum albumin concentrations were determined using a colorimetric assay, with normal range designated as 35–50 g/l. The CRP levels were determined using an enzyme-linked immunosorbent assay (ELISA). Levels below 8.0 mg/l were considered to be normal while levels ≥ 8.0 mg/l were considered to be elevated.

The majority of variables used in these analyses were based on data collected at each subject's baseline clinic visit. Usual dietary intake was assessed at baseline using a self-administered, semiquantitative food frequency questionnaire [34]. Subjects were asked to estimate their frequency of consumption of each of the 116 food items, in terms of a typical portion size, over the previous 12 months. Nine frequency categories were available to choose from, ranging from 'never or less than once per month' to 'more than six times per day'. The questionnaire also had sections for recording the brand name, frequency and amount of vitamin supplements taken, a write-in section for foods not listed, and questions on the exact brands and types of fat used for frying, cooking and baking. Completed questionnaires were reviewed and coded by a trained researcher and entered into computer data files. Estimates of food intake and total intake (food and supplements combined) of vitamins A and E were determined using the nutrient analysis program developed specifically for the questionnaire [34].

HIV-1 serostatus was determined by enzyme immunoassay (Genetic Systems, Seattle, Washington, USA) and a Western blot test (DuPont Co., Wilmington, Delaware, USA and Bio-Rad, Hercules, California, USA) with interpretation using standard criteria. Subjects were divided into three categories based on their CD4+ cell numbers at study entry: < 500, 500–750 and > 750 cells × 10^6/l. These categories were formed to give approximately equal numbers of men in each category. Those who were missing data on CD4+ counts at their baseline visit (n = 32) were assigned to one of the three categories based on data from subsequent adjacent semi-annual visits. It was possible to estimate the appropriate CD4 category for these individuals since the mean CD4+ cell decline in the cohort was 30 cells × 10^6/l per 6 months.

Body mass index was calculated as weight/height^2. HIV-related symptoms were defined as the presence of

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one or more of the following most commonly reported symptoms lasting a period of 2 or more weeks: persistent/recurring fever > 37.8°C, persistent diarrhoea, oral thrush, persistent fatigue, and/or unintentional weight loss of 4.5 kg. Frequency of alcohol consumption was based on the variable 'how often do you drink now?' which was originally coded into eight categories ranging from 'one to five times/year' to 'at least once/day'. For analyses, this variable was collapsed into two categories: less than or equal to two times per week and more than twice per week. For 23 subjects who were missing this data at their baseline visit, values from their next study visit (approximately 6 months later) were substituted. Subjects were placed into one of two categories based on their smoking status: either non-smokers or regular/occasional smokers.

Treatment variables were obtained from data collected over the follow-up period of the study. For the analyses, two binary treatment variables were created indicating 'ever' or 'never' use of antiretroviral drugs (zidovudine, didanosine, zalcitabine) and *Pneumocystis carinii* pneumonia (PCP) prophylaxis (aerosolized pentamidine, trimethoprim–sulfamethoxazole, dapsone) before the onset of AIDS.

The cutoff date for analysis was 31 December, 1993, slightly more than 9 years after the beginning of the Baltimore MACS. Three outcomes were examined in this study including time from baseline study visit to first AIDS diagnosis, time from baseline to death, and time from baseline to first CD4+ cell count < 200 cells × 10^6/l. AIDS diagnoses were based on the 1987 revision of the Centers for Disease Control surveillance case definition [35]. Mortality data were obtained from physicians' reports, hospital records, and death certificates. CD4+ cell counts were measured at every 6-month follow-up visit.

Statistical methods

Proportions of subjects with low serum vitamin A and vitamin E levels were determined according to cutoffs derived from the laboratory standards. All of the independent covariates were examined for their associations with serum vitamin A and E levels using Student's t tests for continuous variables and χ^2 tests for categorical covariates. Univariate associations between serum micronutrient levels and each of the three outcomes were assessed using the non-parametric Kaplan-Meier product-limit method and the log-rank test. The log-rank test was also used to examine associations between the covariates and disease progression.

Cox proportional hazards models were used to adjust for covariates found to be either associated with serum micronutrient levels ($P < 0.15$) or independent predictors of progression in the previous analyses. Variables that did not produce significant changes in the model estimates were eliminated using a step-down procedure. Serum nutrient levels were entered into the models as binary variables (low versus adequate levels) and in quartiles. All independent covariates were entered into the models as categorical variables. Separate Cox proportional hazards models were fit for each micronutrient.

Spearman rank-order correlations were used to examine the linear associations between serum nutrient levels and intake from food and vitamin supplements. The Wilcoxon rank-sum test was used to compare median serum nutrient levels between current and past/never users of multi- and single-vitamin supplements.

Results

Descriptive statistics

Table 1 shows the characteristics of the 312 eligible men in the study. The mean age of the study population at baseline was 34 years (range: 30–65). The mean CD4+ cell count was 643 ± 314 cells × 10^6/l with only 11 men (4%) having CD4+ cell counts below 200 cells × 10^6/l. Although most of the men (81%) were in the asymptomatic stage of their infection, 55% had elevated serum CRP levels (≥ 8.0 mg/l) and 71 (23%) had low serum albumin levels (< 35 g/l).

Over the 9-year follow-up period, 163 men (52%) progressed to AIDS, 162 (52%) died, and 180 (58%) reached a CD4+ cell count of lower than 200 cells × 10^6/l. First AIDS diagnoses were predominantly due to PCP (35%) and Kaposi's sarcoma (25%), followed by *Candida albicans* (8%) and *Mycobacterium avium-intracellulare* complex (6%). Wasting syndrome and dementia were the first AIDS diagnoses for seven (4.3%) and five (3.1%) of the subjects, respectively. Eighty-one per cent (n = 132) of the deaths were attributed to AIDS.

Table 2 summarizes the serum and dietary intake levels of vitamins A and E for the 311 subjects. Mean and median serum levels were within the normal range for both vitamins. Thirty-one subjects (10%) had serum retinol levels below 1.40 μmol/l, and only seven (2%) had levels below 1.05 μmol/l. Twenty-nine per cent of subjects had total intakes of vitamin A (including vitamin supplements) below the recommended dietary allowance (RDA), and 27% had total vitamin E intakes below the RDA.

Univariate analyses

Subjects with low vitamin E levels (< 11.6 μmol/l) were more likely to be non-whites (χ^2 *P*-value = 0.02), not have a college degree (χ^2 *P*-value = 0.007), and not to have used antiretroviral drugs before the onset of AIDS (χ^2 *P*-value = 0.07).

Table 1. Characteristics of 312 HIV-1 seropositive homo-/bisexual men participating in the Baltimore/Washington, DC site of the Multicenter AIDS Cohort Study, 1984.

Characteristic	Frequency n	Frequency %
Demographics (at baseline)		
Age (years)		
< 30	67	22
30–40	194	62
> 40	51	16
Race/ethnicity		
White, non-Hispanic	269	86
Other	43	14
Education level		
College degree	188	60
No college degree	124	40
Alcohol consumption (frequency)		
≤ 2 times/week	171	55
> 2 times/week	141	45
Cigarette smoking		
Non-smoker	194	62
Regular/occasional smoker	118	38
Health status (at baseline)		
HIV-related symptoms*		
No symptoms	254	81
Symptomatic	58	19
CD4+ T cells × 10^6/l		
< 500	118	38
500– 50	97	31
> 750	97	31
Body Mass Index† (quartiles)		
< 21.5	80	26
21.5–22.7	78	25
22.8–24.4	76	24
≥ 24.5	78	25
Serum albumin (g/l)		
< 35	71	23
≥ 35	241	77
C-reactive protein (mg/l)		
< 8.0	141	45
≥ 8.0	169	55
Treatment		
Antiretroviral therapy‡		
Not used before AIDS	165	53
Used before AIDS	147	47
PCP prophylaxis§		
Not used before AIDS	236	76
Used before AIDS	76	24

*Presence of fever > 37.8°C, diarrhoea, persistent fatigue, thrush, or unintentional weight loss ≥ 4.5 kg. †BMI = weight (kg)/height (m)^2. ‡Zidovudine, didanosine or zalcitabine use at any time during follow-up period. §Aerosolized pentamidine, trimethoprim-sulfamethoxazole, or dapsone used during follow-up period. PCP, *Pneumocystis carinii* pneumonia.

No significant difference was observed in risk of AIDS progression between low versus adequate serum vitamin E levels in univariate analyses (Table 3). There was also no significant difference in risk of AIDS progression when comparing those in the lowest quartile of serum vitamin A (< 1.82 μmol/l) with those in each of the upper three quartiles. When vitamin E levels were examined by quartiles, however, a monotonic decrease in risk of progression to AIDS was observed with increasing serum vitamin E levels. The risk of progression to AIDS in the highest quartile of vitamin E level was 35% lower than the risk in the lowest quartile [(relative hazard (RH), 0.65; 95% confidence interval (CI), 0.42–1.02)]. In Kaplan-Meier analyses, median AIDS-free time for subjects in the highest quartile of serum vitamin E level was approximately 1.5 years longer than those in the lower three quartiles combined (log-rank *P*-value = 0.07). There was also an approximate 1 year difference in median survival time between those in the highest quartile of vitamin E level compared to the lower three quartiles combined (log-rank *P*-value = 0.10). No differences were observed in the proportions of subjects whose CD4+ cells declined to < 200 cells × 10^6/l for either vitamin A or vitamin E (data not shown).

Multivariate analyses of serum nutrient levels and HIV-1 disease progression

In order to examine the independent effect of serum nutrient levels on HIV-1 disease progression, Cox proportional hazards models were used to adjust for known and putative confounders. Variables which were associated with AIDS diagnosis, mortality, CD4+ cell decline and/or serum micronutrient levels ($P < 0.15$) were entered as covariates into the Cox models. These included HIV-1 related symptoms, CD4+ cell count, age at baseline, serum albumin, serum CRP use of antiretroviral therapy before AIDS, and frequency of alcohol consumption. Serum albumin and CRP levels were subsequently dropped from the Cox models as they did not produce significant changes in the model estimates for any of the three outcomes.

Older age was strongly and independently associated with an increased risk of progression to AIDS, mortality and CD4+ cell decline. Higher CD4+ cell counts,

Table 2. Summary of serum micronutrient levels and total nutrient intake (from food and supplements) in 311 HIV-1 seropositive homo-/bisexual men participating in the Baltimore/Washington, DC site of the Multicenter AIDS Cohort Study, 1984.

	Mean ± SD	Median (IQR)	Inadequate (%)	Cutoff for inadequacy
Serum nutrient level				
Vitamin A (μmol/l)	2.62 ± 1.08	2.45 (1.82–3.15)	10*	1.36§
Vitamin E (μmol/l)	19.4 ± 11.7	17.2 (12.3–23.5)	22*	11.6§
Total nutrient intake†				
Vitamin A (IU/day)	7405 ± 8562	4739 (1700–8572)	29‡	3330‖
Vitamin E (mg α–TE/day)	133 ± 281	17.9 (8.8–72.9)	27‡	10‖

*Percentage below normal. †Data available on 271 subjects. ‡Percentage below RDA. §Lower limit of normal. ‖RDA, recommended dietary allowance. IQR, Inter-quartile range; α-TE, α-tocopherol equivalents.

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Table 3. Cox proportional hazards models for serum micronutrient levels in 311 HIV-1 seropositive homo-/bisexual men in the the Baltimore/Washington, DC site of the Multicenter AIDS Cohort Study, 1984-1994. Outcome is time to first AIDS diagnosis.

Serum nutrient levels	Number of events / total	Crude relative hazard (95% CI)	Adjusted* relative hazard (95% CI)
Low versus adequate:			
Vitamin E			
≥ 11.6 μmol/l	128 / 242	1.00	1.00
< 11.6 μmol/l	35 / 69	1.22 (0.84–1.78)	1.11 (0.76–1.63)
Quartiles			
Vitamin A (μmol/l)			
Q1: VitA < 1.82	42 / 76	1.00	1.00
Q2: 1.82 ≤ VitA < 2.45	44 / 80	0.79 (0.52–1.21)	1.13 (0.73–1.75)
Q3: 2.45 ≤ VitA < 3.15	37/ 78	0.69 (0.44–1.07)	0.94 (0.60–1.47)
Q4: VitA ≥ 3.15	40/ 77	0.75 (0.49–1.15)	0.73 (0.46–1.16)
Vitamin E (μmol/l)			
Q1: VitE < 12.3	40 / 76	1.00	1.00
Q2: 12.3 ≤ VitE < 17.2	43 / 79	0.91 (0.59–1.40)	1.01 (0.65–1.56)
Q3: 17.2 ≤ VitE < 23.5	43 / 78	0.85 (0.56–1.32)	0.97 (0.63–1.50)
Q4: VitE ≥ 23.5	37 / 78	0.65 (0.42–1.02)	0.66 (0.41–1.06)

*all models adjusted for the following covariates: HIV-related symptoms, CD4+ cell count, age at baseline, use of antiretroviral therapy before AIDS, and frequency of alcohol consumption. CI, Confidence interval; VitA, vitamin A.

more frequent alcohol consumption, and use of antiretroviral therapy before the onset of AIDS were all significantly and independently associated with a decreased risk of progression to AIDS, mortality, and CD4+ cell count decline. The presence of HIV-1-related symptoms at baseline was significantly associated with an increased risk of progression to AIDS and CD4+ cell decline, but not with mortality.

The last column of Table 3 shows the adjusted hazard ratios comparing low versus adequate serum Vitamin levels, and quartiles of serum vitamin A and E levels. For quartiles of vitamin A, there was no apparent relationship between serum levels and progression to AIDS. For vitamin E levels, however, the risk of AIDS remained lower for the highest quartile compared with the lowest quartile (RH, 0.66; 95% CI, 0.41–1.06). This effect was again statistically significant when comparing the highest quartile of serum vitamin E with the remainder of the cohort (RH, 0.67; 95% CI, 0.45–0.98). Similar trends, although not statistically significant, were observed for each vitamin when survival was used as the outcome. In these survival models, we also adjusted for use of antiretroviral therapy after the onset of AIDS, but observed no significant changes in the RH. Furthermore, no significant trends were observed when CD4+ cell decline to < 200 cells × 10^6/l was used as the outcome (data not shown).

Association between serum nutrient levels and self-reported nutrient intake

Data concerning food and supplemental intake of vitamins A and E were available for 271 of the 311 subjects. Those who completed and returned the self-administered food frequency questionnaire were more likely to be non-smokers (χ^2 *P*-value = 0.001), to have a college degree (χ^2 *P*-value = 0.002), and to be older (Student's t test *P*-value = 0.02) than those who did not return the questionnaire. There were no differences in those who did and did not return the questionnaire with respect to the other independent variables, AIDS progression, mortality or CD4+ cell decline.

Spearman's correlations were weak and non-significant between serum vitamin A levels and vitamin A intake from food alone ($r = -0.02$, $P = 0.72$) and from food and supplements combined ($r = 0.02$, $P = 0.74$). Whereas the correlation between serum vitamin E level and vitamin E intake from food alone was weak ($r = -0.06$, $P = 0.29$), the correlation between serum vitamin E level and total vitamin E intake was stronger and statistically significant ($r = 0.30$, $P = 0.0001$).

Table 4 shows the median serum micronutrient levels according to use or non-use of single and/or multi vitamin supplements. Subjects who reported current use of multivitamin or single vitamin A supplements (n = 167) had only a slightly higher median serum vitamin A level than the 104 past/never users. However, current users of multivitamin or single vitamin E supplements (n = 170) had a significantly higher median serum vitamin E level than past/never users (n = 101).

Table 4. Median serum micronutrient levels by use of oral vitamin supplements for 272 HIV-1 seropositive homo-/bisexual men in the the Baltimore/Washington,DC site of the Multicenter AIDS Cohort Study, 1984.

	Vitamin supplements		
Serum nutrient	Current use*	Past/Never use	Wilcoxon *P*-value
Vitamin A (μmol/l)	2.53	2.42	0.20
Vitamin E (μmol/l)	20.1	14.0	0.0001

*Current use of multivitamin and/or single vitamin supplements.

Discussion

We examined the associations of serum levels of vitamins A and E with three key outcomes in the course of

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HIV-1 disease progression: development of first AIDS diagnosis, CD4+ cell decline to < 200 cells × 10^6/l, and mortality. The results of this study showed that subjects in the highest quartile of serum vitamin E levels (≥ 23.5 μmol/l) had a 35% decrease in risk of progression to AIDS compared with those in the lowest quartile, after adjusting for several covariates. A dose–response relationship was evident in univariate analyses. Similar trends were observed for both vitamins when mortality was used as the outcome, but serum levels of neither vitamin were associated with CD4+ cell decline to < 200 cells × 10^6/l.

Previous studies have measured serum levels of vitamin E in HIV-1-infected patients, but ours is the first study to demonstrate a link between increased serum tocopherol levels and decreased risk of progression to AIDS. The prevalence of overtly or marginally low serum vitamin E levels has been documented to range between 8% and 20% in studies of HIV-1-positive individuals [14–16,36–38]. None of these studies have examined the consequences of low serum vitamin E levels on HIV-1 disease progression nor have the mechanisms leading to vitamin E depletion during HIV-1 infection been clearly elucidated. In this study we found that 22% of our population had low concentrations of serum vitamin E (< 11.6 μmol/l), but we found no apparent relationship between low vitamin E levels and risk of progression to AIDS. Instead, we found that subjects in the highest quartile of serum tocopherol levels (≥ 23.5 μmol/l) demonstrated a significant decrease in risk of progression to AIDS compared with those in the lower three quartiles combined.

The effects of various states of vitamin E nutrition on immune function and the potential benefits of vitamin E therapy in HIV-infected patients have been reviewed in several published articles [12,13,39]. The majority of evidence put forth by these reviews suggests that vitamin E is not only necessary for proper functioning of the immune system, but also has important immunostimulatory properties. Studies in both humans and animal models have shown that vitamin E supplementation, in approximately two- to 10-fold excess of the recommendations, significantly increases humoral and cell-mediated immune responses to antigens and enhances phagocytic functions [12]. More specifically, high doses of vitamin E along with a nutritionally adequate diet has been repeatedly shown to enhance *in vitro* and *in vivo* antibody production, phagocytosis, lymphoproliferative responses, and resistance to viral and infectious diseases [13]. Studies in the elderly have examined the effects of vitamin E supplementation on immune dysfunctions associated with the ageing process [9,40]. Daily supplementation of healthy elderly individuals with 800 mg dl-α-tocopheryl acetate for 1 month significantly improved delayed-type hypersensitivity reactions and enhanced *in vitro* response to the mitogen, concanavalin A [9]. Other epidemiological studies in the elderly have found lower incidences of cancer and infectious diseases in those who maintain high plasma vitamin E levels [40,41].

In a recent study [25], the effects of dietary vitamin E supplementation on immune dysfunctions were examined in mice with murine AIDS, a retrovirus-induced infection similar to human AIDS. In this study, infected mice treated with vitamin E showed an increase in secretion of interleukin (IL)-2 and interferon (IFN)-γ, and an improvement in mitogenesis of splenocytes and natural killer cell activity compared with retrovirus-infected, but non-treated mice. At the same time, vitamin E supplementation was associated with a reduction in levels of IL-4, IL-5, IL-6, and tumour necrosis factor (TNF)-α, cytokines which were elevated in non-treated mice which had murine AIDS. This suggests that vitamin E supplementation may serve to normalize the immune abnormalities observed in murine retrovirus infection. Whether or not these results can be extrapolated to human HIV-1 infection, however, remains unclear.

Another mechanism by which vitamin E enhances immune functions is through its antioxidant properties. Rapidly proliferating cells of the immune system are highly susceptible to peroxidative damage by free radicals, peroxides and superoxides [12]. Vitamin E acts as a free radical scavenger and prevents the lipid peroxidation of cell membranes. Vitamin E also modulates the production of prostaglandin E_2 (PGE_2) in the body. Elevated levels of PGE_2 decrease the production of IL-2, a cytokine critical for the growth and differentiation of T and B lymphocytes, and increases the differentiation of PGE_2-receptor-bearing T-cells into T-suppressor cells. PGE_2 also inhibits activation of natural killer cells, which are a major source of IFN-γ, another important cytokine in the host defence system. It is believed that downregulation of PGE_2 by vitamin E serves to increase the production of IL-2 and IFN-γ, thereby stimulating the immune system. Other proposed actions of vitamin E on the immune system are through its direct interaction with macrophages, causing upregulation of IL-1 and IL-2. Vitamin E is also believed to decrease levels of TNF-α through its antioxidant properties. Thus, in addition to its role as a free-radical scavenger, the impact of vitamin E on the regulation of various cytokines in the immune process is believed to play a major role in inhibiting HIV-1 replication.

The results of our study support the hypothesis that these immune-enhancing effects of vitamin E may delay the onset of AIDS in HIV-1-infected individuals. The strong correlation between vitamin E supplementation and increased serum vitamin E levels in our subjects suggests that oral vitamin E therapy may be beneficial in slowing progression to AIDS, even in nutritionally-replete populations.

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Vitamin A is essential for many aspects of immune function, including both B- and T-cell growth and function. In animal models, vitamin A deficiency has been associated with diminished resistance to infections [42], reduced antibody responses to diphtheria and tetanus toxoids [7], and decreased lymphocyte responses to mitogens [7,43]. In humans, the morbidity and mortality of many infectious diseases (e.g. measles, pneumonia and diarrhoea) have been associated with low vitamin A status [8,44–46]. Studies of serum vitamin A levels in HIV-1-infected populations have found that levels below 1.05 μmol/l are commonly observed, with prevalence generally ranging from 10–20%. Sémba *et al.* [18] showed that low serum vitamin A levels in HIV-1-infected mothers were significantly associated with a three- to fourfold increase in rates of transmission of HIV-1 to their infants and that HIV-1-infected mothers whose infants died before reaching 1 year of age had the lowest serum vitamin A levels. In another study by Semba *et al.* [19], low serum vitamin A levels (< 1.05 μmol/l) were significantly associated with a four- to fivefold increase in risk of mortality from AIDS and infections in a population of adult injection drug users. Since biological function is thought to be compromised when serum retinol levels fall below 1.05 μmol/l [8], we did not have large enough numbers to examine associations with HIV-1 disease progression in this lower range. The vast majority of our subjects had retinol levels in the high-normal range (median = 2.44 μmol/l) so it is not surprising that we found no relationship with disease progression. These data, taken with other studies of retinol status in HIV-1 and other infectious diseases suggest that the risk only significantly increases in those with frank or marginal deficiency [17–19]. Furthermore, given individuals with increased serum CRP levels, we still had a vitamin A-replete population, which shows that better vitamin A nutriture can protect against drops in serum retinol into the deficient ranges seen in the acute phase response.

As might be expected since plasma vitamin A levels are homeostatically controlled and do not tend to fluctuate with dietary intake, we found little association between serum vitamin A levels and vitamin A intake from food and/or supplements in our subjects. In a previous study of dietary intake in this population [20], we found that high vitamin A intakes were not associated with a decreased risk of progression to AIDS, wheras moderate intakes were associated with an approximate 40% decrease in risk of AIDS progression.

We compared serum nutrient levels from our population with those from studies of other HIV-1-infected populations in order to determine if concentrations fell within the range of those measured from fresh sera of comparable populations. The mean vitamin A level in our study population (2.62 ± 1.08 μmol/l) was, in fact, higher than the mean vitamin A levels found in most other published studies of HIV-1-seropositive populations [14–18,22,38,47,48]. The mean vitamin A levels in these studies ranged from 0.91 μmol/l to 4.54 μmol/l, with only one study [16] reporting vitamin A levels above the mean level found in our subjects. The mean vitamin E level in our study population was 19.4 μmol/l, which is in the range reported by most studies [14–16,21–23,38,49].

In conclusion, the results of this study suggest that elevated serum levels of vitamin E were associated with a subsequent decrease in risk of progression to AIDS and mortality. High serum vitamin E levels were strongly associated with current use of multi- or single-vitamin E supplements, indicating that vitamin E supplementation may be beneficial in slowing the risk of progression to AIDS. Higher serum vitamin A levels were not associated with intake of vitamin A supplements in this study, however. These data suggest that vitamin E supplementation trials should be considered in HIV-1 infected individuals given the non-toxic nature of the vitamin, its potential therapeutic effects, and the relative sensitivity of serum levels to supplementation.

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IRON DEFICIENCY AND ANEMIA

Serum Ferritin, Erythrocyte Protoporphyrin and Hemoglobin Are Valid Indicators of Iron Status of School Children in a Malaria-Holoendemic Population[1-4]

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ABSTRACT In many African populations, the prevalences of both iron deficiency and malarial infection exceed 50%. The control of iron deficiency anemia is of urgent public health importance, but assessment of iron status in these contexts has been controversial because of the effects of malarial disease on common iron status indicators. We assessed iron status in 3605 school children in Zanzibar by measuring hemoglobin, erythrocyte protoporphyrin (EP) and serum ferritin concentrations. Malaria parasitemia was quantified by counting against leukocytes. Iron deficiency was highly prevalent: 62.4% of hemoglobin concentrations were <110 g/L, 59.7% of EP values were >80 μmol/mol heme, and 41.5% of ferritin concentrations were <12 μg/L. Prevalence of *Plasmodium falciparum* parasitemia was 60.6%, but <1% of children had densities above 5000 parasites/μL blood. Neither hemoglobin nor EP concentration was associated with malaria parasite density, but prevalence of abnormal values increased by ≤25% with parasite density. Erythrocyte protoporphyrin and hemoglobin were strongly inversely related regardless of parasite density. The relationship of EP to hemoglobin was slightly attenuated when parasite density exceeded 1000 parasites/μL blood. Ferritin rose by 1.5 μg/L per 1000 parasites/μL for parasite densities >1000 parasites/μL, but the relationship of ferritin to hemoglobin or EP was strong even when parasite densities exceeded this cutoff. The population prevalences of iron deficiency were not significantly biased by malarial infection. In this population of school children, iron status assessment using these indicators was not seriously influenced by malarial infection. We hypothesize that these indicators perform reliably in populations in which malarial infection is infrequently associated with disease; namely older children and adults in holoendemic environments. J. Nutr. 127: 293–298, 1997.

KEY WORDS: • *humans* • *iron deficiency* • *malaria* • *Plasmodium falciparum* • *hemoglobin* • *ferritin* • *protoporphyrin*

Iron deficiency is probably the most common form of malnutrition in the world, affecting more than half of the women and children in developing countries (DeMaeyer and Adiels-Tegman 1985). In many regions where iron deficiency is of great public health importance, malaria is endemic and the majority of the population harbors subclinical infections. In Africa alone, an estimated 275 million people are infected with this parasite (Najera et al. 1993). Malaria and other infections have complex effects on iron metabolism that may affect the interpretation of hemoglobin, erythrocyte protoporphyrin (EP) and serum ferritin (Baynes et al. 1986, Brabin 1992, Hastka et al. 1993), commonly used indicators of iron deficiency. Although these effects have been described in studies of individuals acutely ill with malaria (Adelekan and Thurnham 1990, Ayatse and Ekanem 1994, Phillips et al. 1986), there is a need for practical guidance regarding the use of indicators to assess iron deficiency in apparently healthy people in malaria-endemic environments.

The spectrum of iron status covers several well-defined stages, ranging from adequate iron storage to depleted iron stores, iron-deficient erythropoiesis, and iron deficiency anemia (Bothwell et al. 1979). To characterize the iron status of a population or to monitor changes in status, it is desirable to use a combination of indicators that provides information about this entire spectrum. Serum ferritin is a particularly useful indicator of iron status, because it is linearly related to iron

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TABLE 1

Expected relationships among iron status indicators during iron deficiency and as a consequence of malaria pathology

Process	Effect	Expected change in indicators[1]		
		Hb	EP	SF
Iron deficiency	Loss of iron stores, accumulation of iron-poor heme, decrease in hemoglobin	↓	↑	↓
Malaria				
Acute phase response to infection	Alterations in indicators unrelated to true iron status	↓	↑	↑
Hemolysis	Recycling of iron, increase in iron stores, stimulation of erythropoiesis	↓	↑	↑
Dyserythropoiesis	Suppressed heme production despite anemia, increase in iron stores	↓	↓	↑
Iron loss in urine or iron trapping in malarial pigments	Decreased body iron stores	↓	↑	↓

[1] Hb = hemoglobin, EP = erythrocyte protoporphyrin, SF = serum ferritin.

stores when stores are present (Cook et al. 1974). Erythrocyte protoporphyrin is a precursor to heme in red cell production. Elevated EP is a sensitive indicator of iron-deficient erythropoiesis (Langer et al. 1972), because EP accumulates when iron is limiting. Hemoglobin concentration is used to define anemia, the end stage of iron deficiency (Anonymous 1989).

The effect of malaria on iron metabolism is not fully understood, but distinct changes in iron metabolism occur during malarial infection that may affect iron status indicators and their interrelationships (Table 1). Malarial disease causes destruction of red blood cells while suppressing erythropoiesis, resulting in a profound anemia (Phillips et al. 1986). These processes would shift iron out of heme toward storage forms. As long as heme iron from destroyed erythrocytes is effectively recycled, these processes would not alter total body stores of iron but would certainly alter the expected relationships among iron status indicators. Malaria may also cause loss of functional body iron, through immobilization in the form of hemazoin (malarial pigments) and through urinary excretion (Brabin 1992). This would decrease iron stores and potentially induce iron deficiency. The effect of the acute phase response to infection may overlap malaria-specific changes in iron metabolism (Baynes et al. 1986). During illness, serum iron concentrations fall as iron is apparently redistributed to storage sites (Elin et al. 1977). As a consequence, hemoglobin falls and EP rises, mimicking the anemia caused by iron deficiency, but ferritin is elevated considerably. In general, on the basis of expected changes in iron status measures, hemoglobin measures would overestimate and ferritin measures would underestimate the prevalence of iron deficiency in a malaria-endemic population.

To determine whether malarial infection influenced indicators of iron deficiency in asymptomatic children, we examined relationships among hemoglobin, EP, ferritin and malaria parasitemia in 3605 children attending schools on Pemba Island, Zanzibar, Tanzania. Coastal East Africa, including the islands of Zanzibar, has the highest malaria endemicity in the world. *Plasmodium falciparum* malaria is holoendemic, that is, parasitemia is highly prevalent but of low density, and transmission is relatively stable, with little seasonality of prevalence of infection (Marsh 1992). This article will specifically address the following questions regarding the relationships of iron status indicators and malaria parasite density in these children: *1*) Is hemoglobin, EP or ferritin or the prevalence of iron-deficiency anemia associated with the level of malaria parasitemia? *2*) Does malaria parasitemia obscure the expected relationships among these indicators of iron status? *3*) Does malarial infection bias estimates of the population prevalence of iron deficiency or iron deficiency anemia using these indicators?

MATERIALS AND METHODS

Study population. This survey was conducted in March through May 1994 on Pemba Island, the smaller of the two islands of Zanzibar, just off the east coast of mainland Tanzania. We randomly selected 12 primary schools from the 72 schools on Pemba Island. The randomization was stratified by the four districts of Pemba Island, so that the 12 study schools represent all parts of the island. From these schools, 76 morning classes of standards 1–4 were invited to participate in the survey. A total of 3605 children were enrolled in the survey, constituting 90% of children registered in those classes and approximately 11% of the total school child population on Pemba Island. The research protocol was reviewed and approved by the internal review boards of The Johns Hopkins University, the World Health Organization, and the Ministry of Health of Zanzibar.

Data. Data were collected in the school classrooms by specially trained local staff of the Ministry of Health. Blood samples were collected by venipuncture from 100% of children surveyed. Hemoglobin concentration was determined in the classroom using the Hemocue portable hemoglobinometer (HemoCue, AB, Angelhom, Sweden). The accuracy of the Hemocues was checked daily with a control cuvette provided with the machines. Precision was ±30 g/L. Erythrocyte protoporphyrin was also measured in the classroom using a hematofluorometer (Aviv Biomedical, Lakewood, NJ). This machine was standardized daily using control solutions provided by Aviv Biomedical. Coefficient of variation for the assay was typically 10±14%. Thick and thin blood smears were made for determination of malaria parasitemia. These were fixed and stained with Giemsa, and malaria parasites were counted against leukocytes. Typically, 200 leukocytes were counted; if <10 parasites were seen, the microscopist continued counting up to 500 leukocytes. Parasite counts were converted to parasite densities on the basis of 8000 leukocytes/μL blood (Trape 1985). Malaria species were identified from the thin smear. All infections were *P. falciparum*; in less than 5% of slides, *P. malariae* was also identified. A random 10% subsample of slides were reread by the Malaria Team leader. Agreement between readers was excellent for the presence of malaria parasitemia [(kappa = 0.93, 95% C.I. (0.89, 0.98)], and parasite density measures were also highly reliable with an intraclass correlation of 0.85. The remaining blood was centrifuged and serum was collected. Sera were stored at −10°C for up to 10 wk, transported on liquid nitrogen to Baltimore, and stored at −70°C for up to 6 mo. Ferritin was determined using a fluorescence-linked immunoassay (DELFIA System by Wallac, Inc., Gaithersburg, MD) on 3309 serum samples (91.8% of the total enrollment). A set of Wallac standards was assayed in each assay plate. Coefficient of variation for this assay was <5%.

Statistical analysis. To determine meaningful cutoffs for categorizing malarial infection in these analyses, we examined the scat-

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TABLE 2

Characteristics of study sample

Sex, *% male*	52.2%
Age, *y*	10.5 ± 1.81
Hemoglobin, *g/L*	104 ± 16
Erythrocyte protoporphyrin, *μmol/mol heme*	97 (56, 166)[2]
Serum fettitin, *μg/L*	14.0 (7.2, 27.3)
Malaria parasites/μL blood, *% frequency*	
0	39.2%
1–999	47.0%
1000–1999	10.0%
2000–2999	2.1%
3000–3999	0.8%
4000–4999	0.3%
≥5000	0.6%

[1] Mean ± SD, n = 3605.
[2] Geometric means ± SD.

terplots of hemoglobin, EP and ferritin against malaria parasitemia. No significant shifts in the mean or variance of these indicators were apparent at infections of <1000 parasites/μL. In subsequent analyses, malarial infection was categorized by thousands of parasites per microliter of blood.

The relationships of hemoglobin, EP and ferritin to malaria parasitemia level are expressed using indicator means and selected cutoffs to define deficiency. Distributions of EP and ferritin were skewed to high values; therefore geometric means are presented. Anemia was defined as hemoglobin <110 g/L. This anemia cutoff is lower than the WHO-recommended cutoff for this age group, but a race-specific anemia criterion (10 g/L lower for blacks) optimizes the screening performance of this indicator to detect iron deficiency (Johnson-Spear and Yip 1994). Ferritin values <12 μg/L were considered to indicate exhausted iron stores, and an EP value of >80 μmol/mol heme was used to indicate iron-deficient erythropoiesis (WHO/UNICEF/UNU 1995). Iron-deficiency anemia was defined as abnormal values for hemoglobin, EP and serum ferritin. Linear trends in mean values were tested by linear regression, and trends in proportions were tested by chi-square test for trend (Snedecor and Cochran 1980).

To evaluate whether the relationships among ferritin, EP and hemoglobin levels differed by low and high parasitemia, we stratified the sample using a cutoff for parasite density of 1000 parasites/μL. Various cutoffs for level of parasitemia were considered, and this cutoff was chosen because it maximized the fit of the model when ferritin or EP was regressed on hemoglobin, level of parasitemia, and their interaction term. Means, SD, and proportions of abnormal values were examined for EP by level of hemoglobin, and ferritin by level of hemoglobin or EP. To determine whether linear trends in these indicators by category were statistically significant for both high and low malaria groups, the indicator was regressed on hemoglobin or EP, malaria level, and their interaction term. The significance level of the interaction term was used to determine whether the relationship between indicators differed by malaria level, with $P < 0.15$ indicating a potentially important difference.

To evaluate the bias caused by malarial infection in evaluating iron status of this population, we compared mean values and the percentage of abnormal values of each indicator in the total sample to those of the children with lower malaria parasitemias. These were tested by Student's t test and chi-square test, respectively (Snedecor and Cochran 1980).

Data were entered using EpiInfo software (Centers for Disease Control and Prevention, Atlanta, GA) and managed and analyzed using Systat statistical software (SYSTAT Inc., Evanston, IL).

RESULTS

Characteristics of the study sample. Several characteristics of the study sample are noteworthy (**Table 2**). First, these first- through fourth-grade school children were relatively old for their school classes. This is typical in Zanzibar, where late school entrance is common. The average age was 10.5 y, with over 75% of children between 9 and 12 y (range 5–19 y). Second, their iron status was very poor; 63% of them were anemic (hemoglobin <110 g/L), 59% had EP values >80 μmol/mol heme, and 41% had ferritin values <12 μg/L. Third, the malaria parasitemia distribution indicated a population with a high prevalence of infection but a low parasite density. Less than 1% of children had >5000 parasites/μL blood, a level that begins to be predictive of clinical disease in highly endemic areas in Africa (Trape et al. 1985).

Association between malaria parasitemia and hemoglobin erythrocyte protoporphyrin and ferritin concentrations. Hemoglobin and EP concentrations were not associated with malaria parasitemia (**Table 3**). The proportion of abnormal values for both hemoglobin and EP showed significant increasing trends with ascending level of malaria parasitemia. Both trends were similar in magnitude, with the prevalence of abnormal values in infected children reaching a maximum of 125% of the prevalence of children with no parasitemia. The highest prevalence of abnormal values was, however, not found in the highest malaria parasitemia group.

Ferritin concentration demonstrated no relationship with parasitemia at parasite densities <1000 parasites/μL blood, but above this cutoff ferritin increased slightly at a rate of 1.5 μg/L per 1000 parasites. This cutoff was used to define high and

TABLE 3

Means and prevalence of abnormal values of iron status indicators by level of malaria parasitemia in children

	Hemoglobin			Protoporphyrin			Serum ferritin			Iron-deficiency anemia[1]	
Malaria parasites/μL	n	g/L	% <110 g/L	n	μmol/mol heme	% >80 μmol/mol heme	n	μg/L	% <12 μg/L	n	%
0	1406	105 ± 16[2]	59[3]	1406	93 (54, 159)[4]	56[3]	1268	14.1 (7.2, 27.7)[4]	40[3]	1267	24
1–999	1678	103 ± 16	65	1678	100 (58, 173)	62	1564	13.3 (7.0, 25.3)	44	1542	30
1000–1999	351	104 ± 16	61	351	99 (59, 165)	63	324	15.1 (7.6, 30.1)	40	324	27
2000–2999	75	104 ± 16	69	75	91 (55, 150)	64	70	17.5 (8.7, 35.4)	27	70	21
3000–3999	27	102 ± 16	74	27	101 (66, 154)	70	27	18.9 (9.1, 39.2)	26	27	22
≥4000	33	104 ± 16	58	33	93 (50, 175)	55	30	26.9 (13.7, 52.8)	13	30	3

[1] Defined as hemoglobin <110 g/L and erythrocyte protoporphyrin >80 μmol/mol heme and ferritin <12 μgL.
[2] Means ± SD.
[3] Significant linear trend by malaria parasite density category ($P < 0.05$).
[4] Geometric means ± SD.

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TABLE 4

The influence of high malaria parasitemia on the relationship of erythrocyte protoporphyrin to hemoglobin (Hb) levels in children

	<1000 malaria parasites/μL			≥1000 malaria parasites/μL		
Hb	*n*	Protoporphyrin	>80 μmol/mol heme	*n*	Protoporphyrin	>80 μmol/mol heme
g/L		*μmol/mol heme*	%		*μmol/mol heme*	%
≥110	1162	77 (52, 114)[1]	42.0	181	78 (53, 114)	45.3
90–109	1492	96 (60, 152)	63.3	233	99 (62, 158)	67.8
<90	428	190 (103, 349)	91.6	72	164 (94, 286)	90.3

[1] Geometric means ± SD. Trends in protoporphyrin (both mean value and % >80 μmol/mol heme) with decreasing Hb were statistically significant at both levels of malaria parasitemia ($P < 0.001$). The increase in protoporphyrin (but not in % >80 μmol/mol heme) associated with the decreasing Hb was greater in those with <1000 parasites/μL (interaction $P < 0.002$).

low malaria parasitemia in subsequent analyses. The prevalence of abnormal ferritin values began to fall at malaria parasite densities >2000 parasites/μL. Both the trend in ferritin concentration and prevalence of abnormal values were significant. The prevalence of iron-deficiency anemia did not show a significant linear trend with malaria parasite density but was dramatically lower in the 30 children with ≥4000 parasites/μL.

Relationships between indicators at low and high parasitemias. Erythrocyte protoporphyrin concentrations, when expressed either as mean values or prevalence of values above 80 μmol/mol heme, showed the expected inverse relationship to hemoglobin (Table 4). This relationship was apparent at both low and high levels of malaria parasitemia. The proportion of children with EP >80 μmol/mol heme was not different between groups with high and low parasite densities. However, EP values associated with the most severely anemic children were not as high in the group with higher parasite densities.

Ferritin was shifted significantly toward higher values when malaria parasitemia was >1000 parasites/μL; this shift is reflected in increases in mean values and decreases in the percentage of children with ferritin <12 μg/L. Nonetheless, the positive association between ferritin and hemoglobin and the negative association between ferritin and EP were strong regardless of malarial infection (Table 5).

These analyses were repeated using malaria parasite density cutoffs of 1 parasite/μL (i.e., any parasitemia) and 2000 parasites/μL (data not shown). The relationships between the indicators was not modified by malaria using any parasite density cutoff.

Effect of parasite density on population assessments of iron deficiency and iron-deficiency anemia. To evaluate the effect of holoendemic malarial infection on population prevalences of iron deficiency and iron-deficiency anemia, we compared the prevalences in the total study sample, representing the general population of school children, to the prevalences in children without parasitemia. The prevalences in the total sample vs. the subgroup with parasitemia were as follows: 62.4% vs. 58.6% for anemia ($P = 0.014$), 59.7% vs. 56.0% for EP ($P = 0.003$), 41.5% vs. 40.5% for ferritin (not significant), and 27.0% vs. 24.2% for iron-deficiency anemia ($P = 0.05$). (For numbers of all children and children without parasitemia, see Table 3.) Although significant due to the large sample size, these differences are not of practical importance when describing the iron status of a population. The mean concentrations of the three indicators in the total sample vs. children without parasitemia were nearly identical: 104 ± 16 vs. 105 ± 16 g/L hemoglobin, 97 (56, 166) vs. 93 (88, 157) μmol protoporphyrin/mol heme, and 14.0 (7.0, 28.0) vs. 14.1 (7.2, 27.8) g/L ferritin, respectively.

DISCUSSION

We have shown that iron status indicators performed reliably in school-age children in a malaria-holoendemic area.

TABLE 5

The influence of high malaria parasitemia on the relationship of serum ferritin to hemoglobin (Hb) and erythrocyte protoporphyrin (EP) levels in children

	<1000 malaria parasites/μL			≥1000 malaria parasites/μL		
	n	Ferritin	<12 μg/L	*n*	Ferritin	<12 μg/L
		μg/L	%		*μg/L*	%
Hemoglobin, *g/L*						
≥110	1038	17.7 (10.0, 31.2)[1]	25.0	163	21.5 (11.8, 39.1)	18.4
90–109	1377	13.1 (7.2, 23.8)	46.4	219	15.2 (8.2, 28.1)	37.4
<90	395	8.1 (4.0, 16.7)	74.7	69	10.6 (4.2, 26.4)	66.7
Protoporphyrin, *μmol/mol heme*						
<80	1130	17.6 (9.6, 32.2)	24.7	167	20.0 (10.4, 38.6)	18.0
80–119	866	14.5 (8.0, 26.1)	39.2	159	17.8 (9.1, 34.6)	29.6
≥120	815	9.1 (5.0, 16.6)	70.7	125	11.0 (5.7, 21.3)	64.8

[1] Geometric means ± SD. Trends in ferritin (both mean ferritin and % <12 μg/L) with increasing level of iron deficiency (by either Hb or EP level) were significant ($P < 0.05$).

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Specifically, *1*) hemoglobin and EP concentrations were not associated with malaria parasitemia, although ferritin concentration did increase slightly with parasitemia when parasite density was >1000 parasites/μL; *2*) expected relationships among indicators were strong regardless of the level of malaria parasitemia; *3*) malarial infection did not modify the relationship among the indicators, except that the elevation in EP with low hemoglobin was slightly attenuated when parasitemia was >1000 parasites/μL; and *4*) malarial infection did not bias population estimates of iron deficiency to a meaningful degree using these indicators.

We cannot evaluate the influence of malarial infection on other indicators, such as transferrin saturation or serum transferrin receptor, because they were not assessed in this survey. A similar evaluation of these indicators in a malaria-endemic population is needed. The three indicators used in this survey were chosen because of their simplicity in the field (in the case of hemoglobin and EP), long-term stability at −20°C (in the case of serum ferritin), relatively low cost to determine, and their complementarity across the spectrum of iron status. Because of these advantages, it is encouraging to find that these indicators are useful in this population.

The influence of malaria on iron status indicators. Although hemoglobin typically falls precipitously (Fleming 1982, McGregor 1982, Phillips et al. 1986) and severe anemia is a common complication and cause of death from acute malaria (Brabin 1992), our data suggest that asymptomatic malarial infection does not much influence hemoglobin concentration. Hemoglobin concentration was not associated with parasitemia, and the increase in prevalence of anemia associated with parasitemia was modest (<25%). Hemoglobin maintained relationships with EP and ferritin that are typically observed during iron deficiency.

Few data are available on the effect of malaria on EP, and we found no published data on EP concentrations during acute malarial disease. In theory, EP values could be elevated in response to infection, as observed in chronic tuberculosis (Langer et al. 1972) and chronic inflammatory neoplastic diseases (Hastka et al. 1993), or decreased due to the dyserythropoiesis that occurs during malaria. In this study, the relationship of EP to hemoglobin was somewhat attenuated in children with higher parasite densities. In contrast to our findings, Schneider et al. (1993) found that mean EP concentration was elevated in children in Togo aged 6 mo to 3 y with parasitemia >3000 parasites/μL. Prevalence of anemia was not associated with parasitemia in those children. The authors noted that the hemolytic breakdown products of heme fluoresce similarly to EP, and speculate that the elevation in EP associated with malaria is artifactual rather than caused by true changes in erythropoiesis. Although the discrepancy of our findings with those of Schneider et al. (1993) is unresolved, the effect of parasite density on EP was not an important consideration when evaluating iron status at the population level in these children.

The most important finding was that the relationship of ferritin to hemoglobin and EP was not affected by malaria parasitemia, even though the magnitude of ferritin values was greater when parasitemia was >1000 parasites/μL. Even in individuals in our population with high parasite densities, ferritin was directly related to hemoglobin and inversely related to EP. Had malaria been a major contributor to anemia, we would have expected the normal relationship between ferritin and degree of anemia to be obscured (Phillips et al. 1986) or reversed (Adelekan and Thurnham 1990), because sicker individuals typically have lower hemoglobin and higher ferritin. Although ferritin values increased slightly with parasite densities >1000 parasites/μL, ferritin was still well below values reported for individuals with acute malaria. For example, the mean ferritin concentration at admission among 23 individuals hospitalized with acute, symptomatic, uncomplicated *P. falciparum* malaria was 1773 μg/L (range 170–10,000 μg/L). Ninety days after initiation of treatment, all values had returned to 150 μg/L or below (Phillips et al. 1986). Only five individuals in our population had ferritin >200 μg/L. At the population level, endemic malarial infection did not affect the prevalence of abnormal ferritin values.

Comparison with other population studies. Others have drawn similar conclusions regarding the reliability of iron status indicators in community surveys of *P. falciparum*-endemic populations. Hercberg et al. (1986) assessed iron status in a randomly selected sample of all community members in a rural district of South Benin, where the prevalence of malaria parasitemia was 96.5%. They reported that hemoglobin concentrations and iron deficiency indicators (ferritin, EP and transferrin saturation) were not related to the density of malaria parasitemia; unfortunately, the evidence for this was not presented in their article. Similarly, in pregnant and nonpregnant women in Papua New Guinea, malaria parasitemia was not associated with significant differences in hemoglobin or EP concentrations (Brabin 1992).

Our findings are in contrast to studies that examined the relationships of malaria infection and iron status indicators in two types of populations: those acutely ill with malaria, in whom iron status indicators are dramatically perturbed (see above), and in young children. Bradley-Moore et al. (1985), in a study of Nigerian children aged 3 mo to 2 y, found that children with parasitemia had around 10 g/L lower hemoglobin and approximately doubled ferritin concentrations compared with their peers without parasitemia. The prevalence of parasitemia in this community was 41%. An age-related effect is also supported by studies in Papua New Guinea (Spencer 1966) and The Gambia (McGregor et al. 1966), in which the association of malaria parasitemia with hemoglobin concentration disappeared with increasing age. Among infants in both studies, children with parasitemia had hemoglobin concentrations 10–20 g/L lower than did their peers without parasitemia. Among children aged 2–4 y, parasitemia was associated with 12 g/L lower hemoglobin concentration in Papua New Guinea but was not associated with lower hemoglobin concentration in The Gambia. Among Papua New Guinean children aged 5–9 y, the association of parasitemia with hemoglobin concentration was slight (a reduction of 3 g/L). Finally, Premji et al. (1995) also found a strong association between anemia (measured by hematocrit) and parasitemia, which decreased with age in 6- to 40-mo-old children in coastal Tanzania, an area expected to have transmission patterns of the parasite similar to those in Zanzibar. Further studies are needed to document, however, whether the effect of parasitemia on other iron status indicators shows a similar disappearance with age in holoendemic environments.

The importance of different patterns of malarial infection and disease. The validity of indicators to assess iron deficiency may be influenced by several factors that dictate how malarial infection will affect a population: parasite species, patterns of transmission, and the susceptibility of population subgroups of interest. Decisions about the use of iron status indicators in *Plasmodium*-infected groups must consider these different situations and population subgroups. Of the four different *Plasmodium* species that cause malaria, each with distinct clinical sequelae, *P. falciparum* predominated in this population. This species causes the most profound changes in iron metabolism

and thus presumably could have the most dramatic effect on iron status assessment.

Different patterns of malaria transmission are broadly categorized as holoendemic, hyperendemic and epidemic, according to intensity and stability of transmission (Najera et al. 1993). The immunity of the population, and thus the probability that infection is associated with disease, differs greatly depending on these patterns. In holoendemic settings such as Zanzibar, parasitemia is prevalent and of high density and splenomegaly is common in infants and young children; however, in adults, malaria parasitemia is prevalent but of low density. Because of the immunity to infection that is acquired in holoendemic settings, the distribution of malarial infection may differ dramatically from that of malarial disease across age groups and seasons (Marsh 1992).

Finally, within a given ecological context, population subgroups have different levels of immunity to the parasite. Immunity, as well as age, is dependent on factors such as general nutritional status and physiologic state, notably pregnancy (Marsh 1992).

We hypothesize that hemoglobin, EP and ferritin are valid indicators of iron status in population groups with relatively high immunity to malaria, but will be less reliable in groups who are less immune. Put in another way, iron status indicators will measure iron status independently of malaria parasitemia in population groups where parasitemia is not strongly associated with disease, which is generally the case among older children and nonpregnant adults in holoendemic environments. If true, this would be important and encouraging news for the assessment of iron deficiency in tropical Africa, where iron deficiency is a prevalent problem among school-age children and adult women. In surveys of iron status in these population groups, it will still be desirable to exclude people who are experiencing fever and those with parasite densities greater than some threshold; Trape et al. (1985) suggest a threshold of 5000 parasites/μL blood. Our data indicate that 5000 parasites/μL would be an appropriate cutoff for hemoglobin and EP. For ferritin, 1000 parasites/μL might be more appropriate, but ferritin would not bias population estimates if the proportion of individuals above such a threshold is relatively small. Further research is required to extend our findings to other vulnerable population groups.

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Viewpoint

Rethinking anaemia surveillance

Rebecca J Stoltzfus

Iron-deficiency anaemia affects about 1·3 billion people[1] and is the most common form of malnutrition in the world. In the past few years, there have been renewed efforts to control iron-deficiency anaemia. At the 1990 World Summit for Children, leaders from more than 159 countries agreed to try to reduce the prevalence of iron-deficiency anaemia by a third by the turn of the century.[2,3] In 1993, the World Bank[4] ranked anaemia as the eighth leading cause of disease in girls and women in developing countries, and concluded that iron-deficiency anaemia could be effectively controlled with existing cost-effective interventions.

Surveillance systems for anaemia are fundamental to public-health decision-making and for monitoring the progress of national and worldwide goals to control anaemia.[5] Effective anaemia surveillance is essential to identify which populations are most in need of intervention and to assess whether programmes are working. As the World Declaration and Plan of Action for Nutrition[6] states, the international community should "strengthen micronutrient surveillance capabilities and activities by devising indicators to monitor . . . strategies for achieving national goals". There is a need for simple indicator cut-offs that enable progress to be tracked and compared across regions, and for criteria on prevalence that identify the most severely affected populations. Such surveillance activities differ from population assessments of the underlying causes or interventions and from the clinical assessments of individuals, for which other methods are recommended.[7-9] Haemoglobin concentration is the key indicator of iron-deficiency anaemia for surveillance purposes, especially since the advent of the HemoCue system (HemoCue, AB, Angelholm, Sweden) which measures haemoglobin concentration within seconds from a drop of whole blood without need of electricity.[10] The current criteria for the assessment of the severity and magnitude of iron-deficiency anaemia in populations are the prevalence of any anaemia and severe anaemia (table 1).

Little progress seems to have been made against iron-deficiency anaemia. Based on surveillance data from 1975 to 1990, the United Nations reported that the prevalence of anaemia worldwide had not declined.[11] Similarly, in sub-Saharan Africa, a region challenged by war and economic instability, and in Southeast Asia, a region characterised by economic emergence and declining rates of other forms of malnutrition, the prevalence of iron-deficiency anaemia has not fallen. A midterm report of the objectives of the World Summit for Children warns that the goal to reduce anaemia by 2000 is "unlikely to be met without a significant acceleration of effort over the next 6 years".[3]

Is it true that efforts to control iron-deficiency anaemia are failing, despite substantial health and economic improvements in some parts of the world? Table 2 shows haemoglobin concentrations for different populations of women. Although all the women are from less developed countries, they were not selected purposively. These data are from studies conducted since 1990 at the Center for Human Nutrition, Johns Hopkins University, Baltimore, USA; none of the studies should be interpreted as nationally representative. The Nepal data were collected in a continuing community-based study[12] in the Terai region, which is representative of the northern Gangetic flood plain of India and southern Nepal. Protein-energy malnutrition, vitamin A deficiency, and hookworm infection[14] are common in women from this region, but there is no programme to provide iron supplements to these women. The Zanzibar data were collected from a rural community-based survey on Pemba Island (Chwaya HM, unpublished observations), where acute malnutrition is common in children, and hookworms and *Schistosoma haematobium* and *Plasmodium falciparum* are endemic.[15] Iron supplements are provided to pregnant women by the Zanzibar Ministry of Health, but coverage is low because of limited tablet supply. The other three areas represent populations where malnutrition is declining and public-health infrastructures are reasonably good. In Peru and Indonesia, iron supplementation for pregnant women has been a long-standing health policy, and tablets are widely available. The Peru data were collected from women who attended antenatal clinics in low-altitude urban areas (Zavaleta N, Fukomoto M, unpublished observations), and the Indonesia data are from a rural community-based study in Central Java.[16] The Shanghai population comprised women who received antenatal care at urban public hospitals.[17] Although this population is poor, acute malnutrition is rare and antenatal care is very good; iron supplements are available but are used by less than 10% of pregnant women.[17]

From all we know about these populations—eg, their dietary adequacy, burdens of infectious diseases, and availability of health services—we would expect to find striking differences in the prevalence and severity of iron-deficiency anaemia. A good indicator should be able to separate these populations according to different degrees

Criterion	Population prevalence category (%)		
	High	Moderate	Low
Any anaemia*	>40	10–39	1–9
Severe anaemia†	>10	1–9	<0·1–0·9

*Haemoglobin concentration <110 g/L in pregnant women or young children, <120 g/L in schoolchildren or non-pregnant women, <130 g/L in adult men.
†Haemoglobin concentrations <70 g/L.

Table 1: **Epidemiological criteria for assessment of severity and magnitude of iron-deficiency anaemia**

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Population	No	% of population with haemoglobin concentrations (g/L)				
		Below anaemia cut-off*	<100	<90	<80	<70
Nepal, 3 months post partum	613	81·4	28·4	13·6	6·3	2·4
Central Java, 3 months post partum	146	71·9	15·1	2·7	2·1	2·1
Zanzibar, not pregnant	583	71·7	26·2	13·0	8·9	5·3
Nepal, pregnant	1052	69·8	40·5	20·8	9·5	4·9
Shanghai, pregnant	829	66·2	25·3	5·5	0·8	0·2
Peru, pregnant	670	44·3	14·5	4·3	<0·1	<0·1

*Anaemia defined as haemoglobin concentrations <110 g/L in pregnant women or <120 g/L in non-pregnant women.

Table 2: **Haemoglobin concentrations in different populations of women**

of risk of iron-deficiency anaemia. However, based on the current criteria for any anaemia, all the populations would be classified as areas with a high prevalence of iron-deficiency anaemia. By contrast, based on the prevalence of severe anaemia, none of the populations would have high rates of anaemia. But this opposition simply does not make sense. Either iron-supplementation programmes and general health indicators have no bearing on the prevalence of iron-deficiency anaemia, or the current criteria do not provide the information needed for the population burden of iron-deficiency anaemia to be assessed accurately. The answer lies in the lower end of the distribution of haemoglobin concentrations. The cumulative distributions in table 2 show the expected differences between the populations. There is only a 1·8-fold difference in the prevalence of anaemia between the populations; however, if haemoglobin concentrations below 90 g/L (moderate-to-severe anaemia) are used as the indicator, there is a 7·7-fold difference between populations. Some comparisons are illuminating. The prevalence of anaemia is similar among non-pregnant women in Central Java and Zanzibar. However, the prevalence of moderate-to-severe anaemia is five times higher in Zanzibar than in Java. The rate of anaemia in pregnant women is similar in rural Nepal and Shanghai, but the prevalence of moderate-to-severe anaemia is 2·5 times higher among Nepalese women than among women from Shanghai.

Severe anaemia (haemoglobin concentration <70 g/L) is closely linked to risk of mortality, but is difficult to monitor because the prevalence rarely exceeds 5–7%, even in populations with a high prevalence of iron-deficiency anaemia. Furthermore, this indicator does not always distinguish between populations with very different haemoglobin distributions—eg, Nepalese and Central Javanese women in the post-partum period (table 2). Changing the cut-off for haemoglobin concentration in anaemia surveillance is such a simple idea that it seems unimportant, but it is not. Let us return to two key objectives of anaemia surveillance: to identify population groups in greatest need of intervention and to monitor progress in anaemia control. The first objective enables donors and governments to use scarce health resources wisely and the second enables them to set goals and see if they are met. How would public-health decision-making be influenced by surveillance of moderate-to-severe anaemia—ie, a haemoglobin cut-off of 90 g/L?

First, public-health resources for anaemia control would be allocated differently. The prevalence of all anaemia or moderate-to-severe anaemia give different indications of the severity of anaemia in the population groups (table 3). The current perception is that anaemia is widespread in all countries. Fund donors, who need to address health issues that are well-defined and amenable to solution, find it difficult to focus their programme efforts on anaemia. Governments of developing countries are overwhelmed with health problems, and may not feel compelled to tackle anaemia when their better-off neighbours report similar rates of anaemia. But, table 3 shows that there are differences in the prevalence of iron-deficiency anaemia between countries and regions, and highlights the need for action in Nepal and Zanzibar.

Second, in developing countries, the prevalence of moderate-to-severe anaemia is more likely to indicate progress in anaemia control if other health improvements are being made. The prevalence of all anaemia in Indonesian women has remained above 50% during the past 30 years, even though progress has been made in most other health indicators, especially nutrition. It would be interesting to know whether the prevalence of moderate-to-severe anaemia has declined or whether it has always been low. I suspect it has declined greatly. Indeed, the lower end of the distribution of a homoeostatically controlled indicator, such as haemoglobin concentration, would be likely to change more rapidly than the upper end. If a successful programme for anaemia control were implemented in Nepal or Zanzibar, the distribution of haemoglobin concentrations might first shift to become like Indonesia—ie, with high prevalence of all anaemia but low prevalence of moderate-to-severe anaemia. This distribution would represent substantial progress, but would not be revealed with surveillance that relied on the prevalence of all anaemia.

If we begin to use the prevalence of moderate-to-severe anaemia for surveillance purposes, it is important not to lose sight of the prevalence of mild anaemia. A survey of haemoglobin concentrations provides data on both mild and moderate-to-severe anaemia. For countries where the prevalence of mild or all anaemia has already fallen below 50%, such as Latin America, the Middle East, and North Africa, the prevalence of all anaemia will measure the lower end of the haemoglobin distribution and is the best indicator for monitoring progress in the control of iron-deficiency anaemia. A suggestion to monitor the prevalence of moderate-to-severe anaemia in regions where the prevalence of iron-deficiency anaemia is high does not imply that there are no health risks associated with mild anaemia. Mild iron-deficiency anaemia affects cognitive capacity in children,[18] increases the risk of preterm delivery in pregnant women,[17,19] and reduces work output in all individuals.[20] Public-health interventions to

Population	No	Prevalence of iron-deficiency anaemia (%)	
		Defined by prevalence of all anaemia*	Defined by prevalence of moderate-to-severe anaemia (haemoglobin <90 g/L)
Nepal, 3 months post partum	613	81	14
Central Java, 3 months post partum	146	72	3
Zanzibar, not pregnant	583	72	13
Nepal, pregnant	1052	70	21
Shanghai, pregnant	829	66	6
Peru, pregnant	670	44	4

*Haemoglobin concentrations <110 g/L in pregnant women, <120 g/L in women who are not pregnant.

Table 3: **Iron-deficiency anaemia in different populations by prevalence of all anaemia and prevalence of moderate-to-severe anaemia**

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control iron-deficiency anaemia are needed in all the populations listed in table 2. But the addition of a lower haemoglobin cut-off for surveillance purposes is important because the health risks associated with moderate-to-severe anaemia are substantially greater; it is within this range that anaemia contributes directly to mortality among women and children.

A helpful analogy is the use of the prevalence of moderate-to-severe stunting or wasting (more than 2 SD below the reference mean) to monitor protein-energy malnutrition worldwide, even though many children who are 1 SD below the reference are malnourished and experience adverse health risks.[21] Similarly, use of low birthweight defined as below 2500 g to monitor rates of low birthweight does not mean that the risk of mortality for a baby who weighs 2600 g is the same as that for a 3300 g baby.[22] Surveillance indicators are chosen because they serve the purposes of surveillance, not because they indicate the point of minimum risk. Despite effective interventions, control of iron-deficiency anaemia has not proved to be easy. We need to intensify our surveillance of anaemia so that the progress we make can be assessed, especially in those countries with a high prevelence of anaemia. A simple change in the haemoglobin cut-offs used for anaemia surveillance would sharpen our focus.

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Hookworm Control as a Strategy to Prevent Iron Deficiency

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The hookworms Necator americanus *and* Ancylostoma duodenale *infect approximately 1 billion people worldwide. The prevalence of hookworm infection increases with age in children, typically reaching a plateau in late adolescence, whereas the intensity of infection may continue to increase throughout adulthood. Hookworms cause intestinal blood loss in amounts proportional to the number of adult worms in the gut. The relationship between hookworm infection intensity and hemoglobin concentration is evident in epidemiologic studies, but may be apparent only above a threshold worm burden that is related to the iron stores of the population. Current hookworm control efforts are focused on reducing infection load and transmission potential through periodic anthelminthic chemotherapy. Several controlled trials have demonstrated a positive impact of anthelminthic treatment on hemoglobin levels, with best results obtained in settings where iron intakes were also increased. Evidence suggests that anthelminthic programs will have modest impacts on iron deficiency anemia in the short term, with greater impacts on more severe anemia. Hookworms are an important cause of anemia in women, who are often overlooked by current helminth control programs. Current WHO recommendations for use of anthelminthics in schoolchildren and women are reviewed. There is a need to clarify whether hookworms are an important etiology of iron deficiency anemia in preschool children.*

Introduction

For decades it has been beyond doubt that hookworms cause blood loss, yet there are few modern examples of hookworm control programs that have been designed with the measured objective of reducing iron deficiency. Nor are there outstanding examples of anemia control programs that have integrated hookworm control as a part of the control strategy. In part this is due to the difficulty of eradicating hookworm infection in populations and the disenchantment with interventions that can reduce the problem but cannot get rid of it. We suspect it is also because scientists are trained to focus within their own discipline, and effective integration of hookworm control and programs to prevent iron deficiency requires collaboration between parasitologists and nutritionists. However, if we are serious about meeting the current global objective of reducing anemia by one-third,[1] hookworm-related blood loss cannot be ignored.

This paper focuses on hookworms and their relation to iron status. However, let us remember that hookworms are not the only helminth—often *Ascaris lumbricoides* and *Trichuris trichiura* are more prevalent than hookworm—and that helminths have other adverse effects in addition to causing iron loss. These effects may include growth retardation, vitamin A deficiency, and cognitive deficits.

We address the following objectives: to review the evidence that hookworm infection is an important etiology of iron deficiency in most populations where hookworms are endemic, to present recent data showing that current anthelminthic therapies can improve iron status, and to summarize current public health recommendations for hookworm control and suggest some ways they could be advanced. Throughout the paper we will draw attention to the importance of hookworms as a cause of iron deficiency in women, a group who have been neglected until recently in most discussions of these issues.

Prevalence and Epidemiology of Hookworm Infection

Hookworms infect approximately 1 billion people worldwide.[2] Prevalence rates range from 10% to 20% in relatively dry, unsanitary areas such as Iran and parts of Pakistan to greater than 80% in rural, unsanitary conditions in the wet, humid tropics.[3] Two species of hookworm, *Ancylostoma duodenale* and *Necator americanus*, are endemic in human populations. *Ancylostoma duodenale* is usually found

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in cooler, drier regions, such as Europe, the Middle East, the Mediterranean, North Africa, Pakistan, and northern India. *Necator americanus* predominates in the Americas, Central Africa, eastern and southern India, Indonesia, and the South Pacific. However, mixed infections are found in many areas of the world, including parts of Latin America.[4]

Both species of hookworm require an infective stage in the human host to complete their life cycles. The transmission of hookworm infection in human populations depends on three factors: the extent of fecal pollution of the soil, the suitability of environmental conditions for egg hatching and larval development, and the extent of contact of contaminated soil with human skin.[5] Contamination of soil occurs when sanitary facilities are not available and community members defecate directly in the soil or when human feces is used as fertilizer for agricultural activities. In warm, humid environments, hookworm eggs in feces hatch and develop into infective larvae, which can live for a period of several days to 1 month[4] before needing to enter a human host to continue their development. The larvae enter the host through the skin, migrate through the circulatory system to the lungs, pass through the alveolar system up the trachea, are swallowed, and finally take up residence in the intestines where they mature into adult worms with a life span of 2–3 years. Adult female worms lay eggs, which pass in the feces and continue the transmission cycle. The presence and number of eggs in the feces also serve as the most practical indicators of hookworm infection in population studies.

People of all ages are susceptible to hookworm infection. However, the prevalence of infection increases with age in children, typically reaching maximum levels at 15–20 years and leveling off in adulthood.[6] Infection intensity follows a similar pattern of increasing with age, but does not necessarily level off during adulthood, often continuing to increase in older adults. Hookworm infection is different in this regard than *A. lumbricoides* and *T. trichiura* infections, which reach maximum prevalence and intensity in the school-age years and then decline in adulthood.

Men often have higher levels of hookworm infection than women, and this is most likely related to gender differences in exposure to contaminated soil.[3] For example, in western and northern Nigeria, women do not do agricultural work in the field, so infection is heavier and more prevalent among men.[7,8] However, in eastern Africa and other areas where women participate in agricultural work, there is no significant difference in prevalence rates between the sexes.[7]

Where hookworm infection is endemic, it is common in pregnant and lactating women. Infection in pregnancy is particularly disastrous to iron status because iron demand is already very high in pregnancy. It is possible that changes in the immune response during pregnancy and lactation may modulate the susceptibility of pregnant and lactating women to hookworm infection, but this has not been adequately investigated.

A characteristic of hookworm and other helminth infections is that a small proportion of individuals carry the majority of the hookworms in the community. For example, a study in West Bengal found that greater than 60% of the hookworms were harbored by less than 10% of the population sampled.[9] This clearly identified distribution pattern of infection in populations has led some to hypothesize that certain "wormy people" are predisposed to heavy infections because of undefined genetic, ecological, behavioral, and social factors. Numerous studies conducted to test this hypothesis have found that the infection intensity prior to anthelminthic treatment is significantly correlated with the infection intensity from reinfection after treatment.[9–12] The causes of this predisposition to hookworm infection have yet to be fully elucidated and could be either behavioral or biological in nature. This epidemiologic characteristic of the disease has led some to suggest that an efficient, economic, and effective hookworm control program would target those individuals predisposed to heavy infection for chemotherapy treatment.[9] However, such a strategy would not address the fact that light or moderate hookworm infection is sufficient to cause anemia in people with low iron stores and intakes. Furthermore, for such a strategy to work in practice, a simple method for accurately identifying "wormy people" within treatment programs would need to be developed.

Effects of Hookworm Infection on Iron Status

Relationship of Hookworm Infection to Iron Loss

The mechanism by which hookworm infection leads to iron deficiency anemia is chronic intestinal blood loss.[13] Adult hookworms attach to mucosa in the upper small intestine, ingesting tissue and blood and changing their feeding site every 4–6 hours.[14,15] Blood is primarily lost when it passes through the hookworm's intestinal tract and is subsequently expelled during feeding, but secondary loss also occurs from bleeding of the damaged mucosa.[4]

Numerous studies have shown that both the worm load and the fecal egg count are strongly correlated with the amount of blood loss.[13,16–19] We measured this relationship in Zanzibari schoolchildren[20] from a sample of 200 chosen to represent the range of hookworm infection intensity in the population (Figure 1). Hookworm infection intensity was measured using fecal egg counts, and gastrointestinal blood loss was measured by determining the concentration of heme in the feces, using the Hemoquant method.[21] This method determines the amount of porphyrins contained in the feces before and after digestion that degrades heme to its porphyrin constituents. The concentration of porphyrin after digestion is used to calculate the total fecal heme concentration, and the ratio of the porphy-

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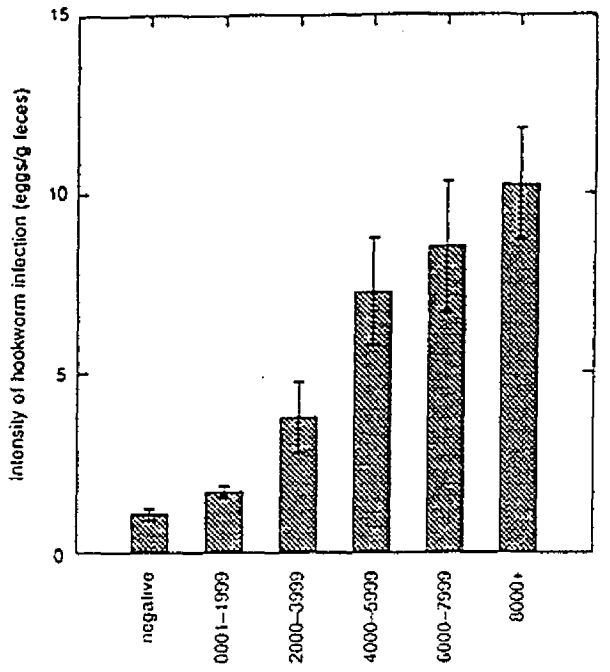

Figure 1. Fecal heme content and hookworm infection intensity in Zanzibari schoolchildren. Bars represent SEM. Increasing trend in fecal hemoglobin is significant ($p < 0.001$). Numbers of children at each ascending level of hookworm eggs per gram of feces are 45, 83, 19, 22, 18, and 16. Reprinted with permission from reference 20.

rin concentration before and after digestion indicates where the blood loss occurred in the intestinal tract. A high proportion of porphyrin in the feces before the digestion process indicates blood loss higher in the intestinal tract. In the case of these samples, a high proportion of fecal heme was already degraded to porphyrin, suggesting blood loss in the small intestine. This is what we would expect from hookworms. A normal Hemoquant value is < 2 mg heme/1 g feces. Like data from earlier experiments using different methods, Figure 1 demonstrates that there is no threshold in the relationship between hookworm infection intensity and intestinal blood loss and that the relationship is approximately linear.

The magnitude of blood loss represented by heavy hookworm infection is substantial. A concentration of 10 mg/g fecal heme represents a daily loss of more than 2 mg iron, more than double the median iron requirement of a healthy school-age child. In children whose fecal heme exceeded 10 mg/g feces, 100% had serum ferritin concentrations < 18 μg/dL, 93% had hemoglobin concentrations < 110 g/L, and 29% had hemoglobin concentrations < 70 g/L.[20] In this sample of children, the predominant hookworm species was *N. americanus*, although some *A. duodenale* was found in about 10% of the children. From studies that have been made of pure infections of the two species, blood loss from *A. duodenale* is 2–10 times higher than that measured in infections of *N. americanus*.[13] Figure 1 thus represents the less pathogenic of the hookworm species.

Table 1. Iron Losses and Requirements for a Typical Woman[a]

Source	Iron Cost (mg/day)
Basal requirement	0.72
Menstruation	0.44
Pregnancy	2.14
Lactation	0.23
Hookworm infection (moderate intensity)	
N. americanus	1.10
A. duodenale	2.30
Other parasitic infections	
T. trichiura (moderate intensity)	0.16
S. haematobium (severe infection)	2.10

[a]Normal requirements from reference 22. Losses from parasites from reference 3.

From this and other studies, the iron loss from a moderate hookworm infection can be compared with other iron requirements (Table 1). A moderate hookworm infection approximately doubles the iron requirement of a woman. Two other parasitic infections are also included in this table for comparison with hookworms. *Trichuris trichiura* is believed to cause a loss of iron about one-tenth that of hookworms, although much less evidence supports this estimate than supports the hookworm figures. *Schistosoma haematobium* infections cause significant urinary iron loss when the infection is severe. However, this parasite is limited to Africa and the Middle East.[3] Malaria, especially *Plasmodium falciparum* malaria, is commonly associated with anemia, but there is no good evidence that malarial infection brings about iron loss. Rather, body iron is dramatically redistributed toward storage forms. Thus, malaria is not a cause of iron deficiency in the same sense as these parasites.

Relationship of Hookworm Infection to Iron Deficiency Anemia

From what we know about hookworms, this parasitic infection should be strongly associated with iron deficiency anemia. Have population studies found this to be the case?

Numerous studies dating back to 1920 have found a significant correlation between hemoglobin levels and worm load and/or fecal egg counts.[13,23] A review of the evidence for the relationship between hookworm infection and iron deficiency anemia also identified some studies where the relationship was not significant. Roche and Layrisse[13] described four conditions necessary to identify this relationship in population studies: a large sample size to account for individual variation, quantitative measurement of hemoglobin levels and worm loads (or egg counts), a wide range of infection levels, and no more than a minimal presence of other anemia-producing deficiencies. In

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Table 2. Relation Between Hookworm Infection Intensity and Hemoglobin Concentration in Nepal and Zanzibar

Hookworm Infection Intensity (eggs/g feces)	Hemoglobin Concentrations (g/L)			
	Nepalese Pregnant Women[a]	Zanzibari Schoolchildren[b]	Zanzibari Adult Men	Zanzibari Nonpregnant Women
0	105 ± 12 (97)[c]	110 ± 13 (215)	129 ± 19 (24)	115 ± 16 (53)
1–1999	102 ± 14 (256)	105 ± 13 (2562)	121 ± 22 (351)	109 ± 18 (375)
2000–3999	100 ± 18 (39)	100 ± 17 (456)	113 ± 28 (72)	105 ± 22 (97)
4000–5999	86 ± 21 (24)	96 ± 19 (128)	104 ± 23 (34)	105 ± 24 (42)
≥ 6000	89 ± 20 (18)	93 ± 23 (67)	96 ± 38 (14)	98 ± 20 (15)

[a] From reference 27.
[b] From reference 28.
[c] Mean ± SD; sample size in parentheses.

most studies finding no significant correlation between hookworm infection and iron deficiency anemia, at least one of these conditions was not met.[13]

Layrisse and Roche's seminal population-based study of hookworm infection in Venezuela[23] documented a highly significant relationship between hemoglobin levels and fecal egg counts. However, a threshold effect was also observed, demonstrating the dependency of this relationship on a minimum level of infection. Hemoglobin levels were significantly lower in women and children with more than 2000 eggs per gram (epg) of feces and in men with more than 5000 epg compared with those with light infections. Some other studies have found the same phenomenon, although the threshold worm load varied from 1000 to 5000 epg.[24–26]

The threshold worm load associated with anemia does not derive from the characteristics of hookworm infection—we have seen that the relationship between hookworm infection intensity and iron loss has no threshold—but rather from the characteristics of anemia as an indicator of iron status. Hemoglobin concentration falls only when iron stores are depleted. The development of iron deficiency anemia from hookworm infection thus depends on three factors: iron intake, iron stores, and the intensity and duration of infection.[7] Iron intake and stores differ significantly between communities and population subgroups. Thus, a light infection might be sufficient to cause negative iron balance in someone with a low iron-bioavailable diet, but another person with a more iron-rich diet would have negative iron balance only if more heavily infected. Women and young children usually have the lowest iron stores, making them particularly vulnerable to developing iron deficiency anemia from chronic blood loss due to hookworm. This is illustrated in the work of Layrisse and Roche[23] in Venezuela, where low hemoglobin levels occurred at a lower threshold worm load in women and children than in men. At the opposite extreme, in a Nigerian population where average iron intake is 21–30 mg/day, iron deficiency anemia was not apparent unless subjects were infected with more than 800 worms.[8]

We have studied the association between hookworm infection intensity and iron deficiency anemia in two regions that have iron-poor diets: Pemba Island, Zanzibar, in East Africa, and the Terai region of Nepal, just across the border from northern India. In Zanzibar, the Ministry of Health has conducted a representative survey of schoolchildren on Pemba Island and a community-based survey of adult men and nonpregnant women. In Nepal, we are continuing to evaluate the iron status and risk factors for iron deficiency in pregnant women in Sarlahi district. In each of these studies, hemoglobin and erythrocyte protoporphyrin concentrations have been determined from venous blood samples, and hookworm infection intensity was assessed by the Kato-Katz method of counting eggs per gram feces.

In each of these population subgroups the relationship between hookworm infection intensity and hemoglobin concentration is very strong (Table 2). In Zanzibari women and children and pregnant Nepalese women, the hemoglobin concentration falls about 5 g/L per 2000 hookworm epg. In Zanzibari men the decline is even steeper, about 8 g/L per 2000 hookworm epg. The iron status of these populations is very poor. Even among the groups with no hookworm infection, mean hemoglobin levels are below normative definitions of anemia. Therefore, the decline in hemoglobin is apparent even at the lowest levels of hookworm infection intensity. In populations like these, individuals have no iron stores to buffer the losses caused by hookworms, and thus the linear intensity-dependent relationship between hookworm infection and iron status

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Table 3. Proportions of Anemia Attributable to Hookworm Infection in Different Population Groups

Population Group	Type of Anemia[a]	
	Iron Deficiency Anemia	Moderate to Severe Anemia
Zanzibari schoolchildren	41%	57%
Zanzibari men	31%	31%
Zanzibari nonpregnant women	19%	56%
Nepalese pregnant women[b]	29%	41%

[a] Iron deficiency anemia is defined as protoporphyrin > 80 μmol/mol heme and hemoglobin < 110 g/L in pregnant women and schoolchildren or <120 g/L in nonpregnant women or < 130 g/L in men. Moderate to severe anemia is defined for all groups as hemoglobin < 90 g/L.
[b] From reference 27.

is readily observed.

Because these data are representative of their respective communities, we can calculate the proportion of anemia in the population group that is attributable to hookworm infection, i.e., the attributable fraction (Table 3). This is analogous to attributable risk,[29] but anemia prevalence rather than incidence is used. These proportions can be thought of as the maximum fraction of anemia that would be prevented if hookworms were removed from the population. Reduction of hookworm burdens will make a sizable impact on the prevalence of iron deficiency anemia, especially moderate to severe anemia, in these groups.

Treatment and Prevention of Hookworm Infection

Surely the eradication of hookworm infection is an appropriate long-term goal for countries and regions where the parasite is now endemic. However, the inability to permanently rid communities of hookworm due to frequent reinfection has contributed to a loss of support for eradication efforts. More recently, the programmatic focus has shifted from eradication to control. The intensity-dependent relationship between hookworm infection and iron deficiency means that reduction of infection intensity alone will help control iron deficiency anemia in communities. Thus, current hookworm control efforts are focused on reducing infection load and transmission potential to reduce morbidity associated with the disease.[5] Periodic anthelminthic chemotherapy, ideally in the context of ongoing sanitation efforts, is the key intervention in current hookworm control strategies.

Anthelminthic Drugs

Several safe and effective anthelminthic drugs are available to treat hookworm infection. The benzimidazoles (albendazole and mebendazole) are broad-spectrum anthelminthics that are increasingly popular for individual and communitywide treatment of hookworm infection. They are effective at substantially reducing the intensity and somewhat reducing the prevalence of infection with either hookworm species.[30-33] The benzimidazoles reduce prevalence and intensity of *A. lumbricoides* infections with >90% effectiveness but are much less effective against *T. trichiura* infections, usually reducing only the infection intensity.[34]

The two benzimidazole drugs, albendazole and mebendazole, differ in terms of dose and cost. The recommended dose of albendazole is 400 mg whereas mebendazole can be taken as a single 500-mg dose or as 200 mg daily for 3 days.[35] Although treatment trials indicate that mebendazole's 3-day dosing schedule is most efficacious,[33,36,37] a single-dose treatment is clearly preferable for public health control programs. A recent trial compared the efficacy of single doses of albendazole and mebendazole and found mebendazole to be almost as efficacious as albendazole in the treatment of hookworms.[38]

Two other drugs may also be used to treat hookworm infections. Pyrantel is effective in reducing the prevalence and intensity of hookworm infections.[5] The dosage is 10 mg/kg body weight for 3 days.[35] The requirement for multiple doses presents logistical problems in public health settings. Levamisole is generally considered to be less effective at curing hookworm infection. However, it does reduce the intensity of infection as measured by egg counts.[39] The recommended dose is 3 mg/kg body weight.[35]

Concern about the safety of anthelminthic drugs for the fetus and pregnant mother has limited efforts to control hookworm infection as a cause of iron deficiency anemia in women. However, the World Health Organization (WHO) recently convened a meeting to address this issue, and it was concluded that "single-dose, oral anthelminthic treatment can also be given to pregnant and lactating women. However, as a general rule, no drug should be given in the first trimester."[40] This statement should allow for new avenues of research on hookworm infection and the importance of its control in women.

Sanitation

Transmission of hookworm infection occurs primarily through physical contact with soil contaminated by human feces.[41] Therefore, safe disposal of human feces is essential for the eradication of hookworm infection. Sanitation measures include the provision of latrines along with training in their use and the appropriate treatment of human waste before use in agriculture. Sanitation control pro-

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Table 4. Results from Controlled Trials of Anthelminthic Treatment for Hookworm Infection: Impact on Hemoglobin Levels

Country	Target Population	Hookworm Prevalence %[a]		Impact on Hemoglobin[b]
		Pretreatment	Posttreatment	
Kenya[c]	Children	83	53	+ 3 g/L after 6 months
Kenya[d]	Children	94	88	+ 3 g/L after 8 months
Kenya[e]	Children	96	44	+ 4 g/L after 4 months
Papua New Guinea[f]	Adult males	100	3	+ 6 g/L after 5 months
India[g]	All	54[h]	—	
1–5 years				+ 5 g/L after 6 months
6–14 years				+ 6 g/L after 6 months
15–44 years, males				+ 3 g/L after 6 months
15–44 years, females				+ 2 g/L after 6 months
≥ 45 years				+ 5 g/L after 6 months

[a]Prevalence rates of the treatment group at baseline and posttreatment.
[b]Impact is defined as the pre-post difference in the treatment group minus the pre-post difference in the placebo group.
[c]From reference 43.
[d]From reference 44.
[e]From reference 45.
[f]From reference 30.
[g]All individuals in the deworming trial had received iron-fortified salt for 6 months prior to deworming and throughout the deworming trial. From reference 46.
[h]Population prevalence from a previous study. From reference 47.

grams aim to reduce or interrupt transmission, prevent reinfection, and gradually reduce worm loads.

Footwear

Use of footwear to protect feet from contact with contaminated soil has been recognized as an important preventive measure against hookworm infection since the 1920s when Smillie documented the association between hookworm infection and plantation work.[41] In a subsequent study in Alabama, Smillie found that the highest prevalence of hookworm in the community was among schoolchildren (99%) who commonly did not wear shoes, and he strongly advocated footwear use as protection from hookworm infection.[42] This highly sensible recommendation is well accepted, although no real evaluations of its effectiveness have been done. Also, debate continues on whether promotion of footwear use is a feasible and effective hookworm control measure given the difficulties inherent in any behavior change strategy. However, this would seem to be a relatively simple behavior change compared with many other public health interventions such as condom use and dietary modification.

Efficacy Trials of Hookworm Control to Improve Iron Status

Several controlled trials have demonstrated a positive impact of anthelminthic treatment on hemoglobin levels (Table 4). Other trials that have not found a positive impact on hemoglobin concentration following deworming have not employed a control group[48] or have had follow-up periods of less than 8 weeks.[49,50] These controlled trials show that deworming can have a positive impact on hemoglobin concentration. It is also apparent that hookworm does not need to be cured in all individuals to benefit iron status. In the Kenyan studies,[43–45] a positive impact on hemoglobin was observed even though the prevalence of hookworm infection was decreased at most by around half. In each of these studies the intensity of infection was significantly reduced among those individuals who remained infected. The results of the trials in Papua New Guinea and India are particularly impressive because the subjects were also provided with increased amounts of dietary iron. In Papua New Guinea[30] the men were newly incarcerated prisoners provided with a nutritionally adequate prison diet, and all participants in the Indian trial were provided iron-fortified salt for 6 months prior to deworming and throughout the deworming trial.[46]

Effectiveness of Hookworm Control Programs to Improve Iron Status

Several regional hookworm control programs have been evaluated for their impact on hookworm infections;[31,51,52] however, evaluations of program effectiveness to improve iron status have been lacking. Until it has been demonstrated that large-scale anthelminthic programs actually do improve the iron status of communities, it will be difficult to garner support for their integration into strategies to control iron deficiency.

We recently completed an evaluation of the Zanzibar school-based deworming program, which is aimed at all primary school children in grades 1–5. The prevalence of hookworm infection in this population and age group is

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more than 90%.[38] So that the Ministry of Health of Zanzibar could obtain a valid estimate of program impact on children's nutritional status, the program was phased in during its first year. In this way, children in schools receiving the program could be compared with children in schools not yet receiving the program. By the second year of the program, all schools received the full program regimen.

The program was implemented by the Zanzibar ministries of Health and Education, and the evaluation was implemented by the Pemba Island Helminth Control Team, Malaria Control Team, and the Nutrition Unit of the Ministry of Health. Out of 72 schools on Pemba Island, 12 were randomly selected for the evaluation. From these schools, 3605 children in grades 1–4 participated in the baseline survey, and 92% of these children were measured again at the 12-month follow-up survey. Of the 12 evaluation schools, 4 received deworming 3 times per year, 4 received deworming 2 times per year, and 4 received no deworming program. Deworming consisted of a single 500 mg dose of generic mebendazole. The cost of this drug was US$0.027 per dose.

In its first year, neither twice-yearly nor thrice-yearly deworming had a significant impact on average hemoglobin concentrations.[53] However, thrice-yearly deworming reduced the incidence of severe anemia during the 1-year period by approximately one half. Deworming also improved children's iron status as measured by serum ferritin and erythrocyte protoporphyrin. As expected from the linear relationship between hookworm infection intensity and intestinal blood loss, children with heavier hookworm infections at baseline benefited most from the intervention.

As these results demonstrate, even in populations where hookworms are highly endemic, the short-term impact of deworming alone on iron deficiency anemia will be modest. If one thinks of a person's iron status as a bucket that needs filling, supplementation is like pouring iron into the bucket, food fortification is like trickling iron into the bucket, and deworming is like partially plugging a hole in the bucket. The impact from deworming alone might be compared to that of a food fortification program, because the impact is incremental and typically small in a 1-year time frame. However, these early results from the Zanzibar program coupled with the pattern of attributable risks of anemia in Table 3 suggest that deworming will have the greatest impact on the left tail of the hemoglobin distribution.

Certainly deworming alone will not solve the problem. The control of iron deficiency anemia in settings like Zanzibar will require integration of multiple interventions. However, deworming is low cost, feasible, and highly desired by communities, and it does improve children's iron status. Deworming will be one essential component of an effective iron deficiency control program for Zanzibar and for other communities where hookworms are an important etiology of iron deficiency.

Recommended Approaches to Hookworm Control

We want to end by reviewing the two current policy recommendations made by WHO with regard to deworming and suggesting ways that they can be further advanced. The first addresses anthelminthic treatment in school health programs,[39] and states: "Treatment without prior individual screening of the whole population is recommended where surveys of school-age children indicate the prevalence of intestinal helminths or schistosome infection exceeds 50%." The stated objective of this policy recommendation is to prevent adverse effects of helminth infections on health, growth, and school performance of children. To date, the prevention of anemia in schoolchildren has not often been an explicit objective of school-based deworming programs. For those of us concerned with the control of iron deficiency, it would be helpful to refine this general recommendation to address the prevalence of hookworm for which treatment of the whole population is likely to bring about some impact on the prevalence of iron deficiency in schoolchildren. It would also be helpful to have some estimate of the benefit to children's iron status that can be expected in a given time frame. One of our current collaborative research objectives is to begin to address these policy questions with the data from Zanzibar.

More recently, a recommendation was made to address the problem of hookworms in girls and women.[40] This recommendation reads: "Since hookworm infections contribute to iron-deficiency anaemia, it is recommended that, in areas where these infections are endemic (prevalence > 20–30%) and where anaemia is prevalent, hookworm control ... be included in strategies designed to improve the health, development and nutritional status of girls and women." It goes on to state that several anthelminthic drugs may be safely given to pregnant women beyond their first trimester. This policy statement, in contrast to the one for school-age children, was specifically motivated by the role of hookworms in causing anemia. The expectation is that anthelminthic treatment, especially combined with supplemental iron, will help prevent iron deficiency anemia and its consequences during pregnancy.

Sri Lanka has made deworming, in addition to iron-folate supplementation, a routine part of its public health antenatal care program. In the program, pregnant women receive a single dose of mebendazole in the second trimester of pregnancy. The mebendazole is produced locally in Sri Lanka for less than US$0.03 per dose. A single evaluation of this intervention has been carried out in 195 pregnant tea plantation workers.[54] The plantation community was chosen for the study because these women are at highest risk for hookworm infection. Women received interventions as they were delivered by the public health service; the study investigators did not control the interventions in any way. Thus, it happened that some women received iron-folate tablets and some did not, but all of the

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women who received mebendazole also received iron-folate tablets. Receiving the combination of mebendazole with iron-folate supplementation was more effective in improving women's iron status during pregnancy than receiving iron-folate supplementation alone.

This is a small study, but the results suggest that antenatal anthelminthic therapy could play an important role in preventing iron deficiency in women of reproductive age. Unfortunately, hookworm infections were not assessed in these women, so this impact cannot be related to the baseline prevalence and intensity of infection in the population. Additional research is needed to refine the recommendation with regard to the endemicity of hookworm infection in populations of women and whether a single treatment is the appropriate regimen in most circumstances.

From a nutrition perspective, it is noteworthy that there are no recommendations for the use of anthelminthic therapies for preschool children, in whom the problem of iron deficiency is particularly prevalent and severe. If anthelminthic therapy can improve the iron status of a 7-year-old schoolchild, can it also benefit a 5-year-old or a 3-year-old? The potential adverse effects of *Ascaris* infection on growth and vitamin A status have been the focus of the discussion of deworming in this age group. But where hookworm infection is endemic, it would be very useful to clarify whether hookworms are an important etiology of iron deficiency in young children and an additional motivation for preschool deworming programs.

Theoretical calculations suggest that hookworms could contribute to iron deficiency in preschoolers. Although hookworm infection intensities are light in young children compared with school-age children and women, a small blood loss in a small child is important. A light infection (1000 epg or 40 worms) of *N. americanus* causes a daily blood loss of around 0.55 mg,[3] equal to the median iron requirement of 0.56 mg for children 2–6 years old.[7] If children in many environments cannot meet this normal requirement, they certainly cannot offset a blood loss that doubles their requirement for absorbed iron. In many countries where targeted fortification efforts are not yet feasible, no interventions exist to address the problem of iron deficiency in young children. Where hookworms are endemic, anthelminthic therapy may be a feasible intervention.

In conclusion, it is important to reiterate that hookworms are not the only helminth and that hookworms may inhibit children's growth and other aspects of development as well as induce iron deficiency. Helminth control programs must be designed to address a broader framework of health concerns than only iron deficiency. Yet at this moment, while there is a global commitment to the control of iron deficiency and a particular enthusiasm for deworming as a public health intervention, it is timely for nutritionists to clarify the importance of hookworms as an etiology of iron deficiency in different population contexts and to evaluate the role of anthelminthic therapy in strategies to control iron deficiency.

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Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms[1–3]

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ABSTRACT Anemia is estimated to affect one-half of school-age children in developing countries. The school years are an opportune time to intervene, and interventions must be based on sound epidemiologic understanding of the problem in this age group. We report on the distribution of iron deficiency and anemia across age, sex, anthropometric indexes, and parasitic infections in a representative sample of 3595 schoolchildren from Pemba Island, Zanzibar. Iron status was assessed by hemoglobin, erythrocyte protoporphyrin (EP), and serum ferritin concentrations from a venous blood sample. Overall, 62.3% of children were anemic (hemoglobin < 110 g/L), and 82.7% of anemia was associated with iron deficiency. The overall prevalence of iron-deficient erythropoiesis (EP > 90 μmol/mol heme) was 48.5%, and the prevalence of exhausted iron stores (serum ferritin < 12 μg/L) was 41.3%. In bivariate analyses, iron status was slightly better in girls than in boys, and was better in children aged 7–11 y than in those older or younger. Hemoglobin but not EP or serum ferritin concentrations were lower in stunted children. Infection with malaria, *Trichuris trichiura*, *Ascaris lumbricoides*, and hookworms were all associated with worse iron status; the association with hookworms was strongest by far. In multivariate analyses, hookworm infection intensity was the strongest explanatory variable for hemoglobin, EP, and serum ferritin. Sex, malarial parasitemia, *A. lumbricoides* infection, and stunting were also retained in the multivariate model for hemoglobin. Twenty-five percent of all anemia, 35% of iron deficiency anemia, and 73% of severe anemia were attributable to hookworm infection; < 10% of anemia was attributable to *A. lumbricoides*, malaria infection, or stunting. We conclude that anthelminthic therapy is an essential component of anemia control in schoolchildren in whom hookworms are endemic, and should be complemented with school-based iron supplementation. *Am J Clin Nutr* 1997;65:153–9.

KEY WORDS Humans, iron deficiency, anemia, schoolchildren, geohelminths, hookworms, malaria, schistosomiasis, ascariasis, trichuriasis

INTRODUCTION

Iron deficiency affects more people in the world than any other form of malnutrition, and its control is a global priority in public health. Iron deficiency anemia results from a variety of causes, including inadequate iron intake, high physiologic demands in early childhood and pregnancy, and iron losses from parasitic infections. The relative importance of these causes, and thus the appropriate strategies for prevention of iron deficiency anemia, differs among populations and age groups.

Iron deficiency anemia is most prevalent and severe in young children and women of reproductive age (1). As a result, these groups have been the focus of most epidemiologic investigations of the problem and its causes. However, iron deficiency anemia is also common among school-age children. In developing regions of the world, the prevalence of anemia in 5–12-y olds is estimated to be 46%, with the highest rates found in Africa (49%) and South Asia (50%) (2). The school-age years are an opportune time to address this problem for several reasons. Iron deficiency impairs children's cognitive abilities, and interventions to prevent and correct iron deficiency may enhance children's learning potential in school (3). Improving the iron status of schoolchildren will also increase their fitness and work capacity (4), and improvements in girls' iron status during the school-age years may help to prevent anemia during their reproductive years. Finally, the school setting offers an ideal distribution system for public health interventions of many types (5), including health education, iron supplementation, and treatment or prevention of parasitic infections.

To develop and target effective interventions to prevent iron deficiency anemia in school-age children, a sound epidemiologic understanding of the problem is needed. As a first step to evaluate a school-based deworming program in Zanzibar, we conducted a baseline survey of iron deficiency anemia and its determinants in schoolchildren. Our objective in this paper was to describe the distribution of iron deficiency and anemia in Zanzibari schoolchildren by age and sex, anthropometric indicators, and parasitic infections. We then used these findings to

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discuss the most promising strategies for control of iron deficiency anemia in schoolchildren of coastal east Africa.

SUBJECTS AND METHODS

Study population

The study was conducted on Pemba Island, the smaller of the two islands of Zanzibar, just off the east coast of mainland Tanzania. The population is predominantly rural, with agricultural activities devoted to subsistance crops and cloves, an exported cash crop. Typical of coastal East Africa, Pemba Island is characterized by intense transmission of *Plasmodium falciparum* malaria, *Schistosoma haematobium*, and geohelminths. Both species of hookworms (*Necator americanus* and *Ancylostoma duodenale*) are endemic, with *N. americanus* predominating (6). The iron bioavailability of the Pembian diet is low. The primary staple food is cassava, with rice being more desired but less affordable. These foods are usually eaten with vegetable sauces and small fish. Meat is always a luxury item, and there are seasonal shortages of vegetables and fish.

The study population comprised first- through fourth-grade schoolchildren of Pemba Island, Zanzibar. This survey was conducted between March and May 1994, and was reviewed and approved by the institutional review boards of The Johns Hopkins University, the Ministry of Health of Zanzibar, and the World Health Organization. To form the study sample, we randomly selected 12 primary schools from the 72 schools on Pemba Island. From these schools, 76 morning classes of standards 1–4 were invited to participate in the survey. A total of 3605 children were enrolled in the survey, constituting 90% of children registered in those classes and ≈11% of the total schoolchild population on Pemba Island. Data were collected in the school classrooms by specially trained local staff of the Ministry of Health. Age and sex data were obtained from 3595 children (99.7% of the sample), and these children were used in the present analyses.

Assessment of nutrition and health status

Children's weight was measured to the nearest 0.1 kg with a battery-powered digital scale (Seca, Inc, Columbia, MD). Height was measured to the nearest millimeter with a wooden stadiometer (Shorr Productions, Inc, Olney, MD). Age was calculated from the birth date on school records, which are based on birth certificates. When the school had no record of age, the child's self-reported age was used.

Iron status and anemia were assessed by hemoglobin, erythrocyte protoporphyrin (EP), and serum ferritin (SF) concentrations. Assessing the iron status of malaria-endemic populations has been a subject of controversy; however, we found that these indicators assessed iron status reliably in this population (7). Blood samples were collected by venipuncture from 100% of children surveyed. The hemoglobin concentration was determined by using the HemoCue hemoglobinometer (HemoCue, AB, Angelhom, Sweden), and the EP concentration was measured with a hematofluorometer (Aviv Biomedical Inc, Lakewood, NJ). An aliquot of blood was centrifuged at $1000 \times g$ for 20 min at room temperature and serum was collected. Sera were stored at −10 °C for ≤ 10 wk, transported on liquid nitrogen to Baltimore, and stored at −70 °C for ≤ 6 mo. SF was determined by using a fluorescence-linked immunoassay (DELFIA System by Wallac, Inc, Gaithersburg, MD) on 3309 serum samples, 91.8% of the total enrollment. Missing data for SF were the result of losses of samples from mishandling or mislabeling in transport to Baltimore, or the result of laboratory error.

Thick and thin blood smears were fixed and stained with Giemsa, and malaria parasites were counted against leukocytes. Typically, 200 leukocytes were counted; if < 10 parasites were seen, the microscopist continued counting up to 500 leukocytes. Parasite counts were converted to parasite densities on the basis of 8×10^9 leukocytes/L blood (8). Malaria species were identified from the thin smear. All infections were *P. falciparum*; in < 5% of slides, *P. malariae* was also identified. A random 10% subsample of slides were reread by the Malaria Team leader. Agreement between readers was excellent for the presense of malaria parasitemia (kappa = 0.93, 95% CI: 0.89, 0.98), and parasite density measures were also highly reliable with an intraclass correlation of 0.85.

For the assessment of helminth infections, containers were distributed to each class and the children were asked to bring a sample of their feces to school the next day. More than 95% of children surveyed returned a fecal sample. These samples were stained the same day and examined within 1 h of staining by using the Kato-Katz method (9). Hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* eggs were counted. If > 10 000 eggs per gram feces (epg) were found for any helminth, the sample was diluted by using a modified Stoll technique (10) and was reexamined. A random 10% subsample of fecal smears was reexamined by the most skilled technician for quality control. Intraclass correlations between staff and quality-control readings were 0.88 for *A. lumbricoides* epg, 0.79 for *T. trichiura* epg, and 0.89 for hookworm epg.

As an indicator of *S. haematobium* infection, urine samples were collected on the day of the survey to test for microhematuria with Hemastix test strips (Ames Laboratories, Elkhart, IN). This procedure screens for urinary schistosomiasis with 69% sensitivity and 89% specificity (11).

Data analysis

Stunting was defined as a height-for-age Z score < -2. These Z scores were calculated by using EpiInfo (Centers for Disease Control and Prevention, Atlanta). An appropriate indicator for wasting in primary school-aged children is not well defined. For children aged > 10 y, the 5th centile of body mass index [BMI, weight (kg)/height squared (m^2)] is recommended whereas for children aged 5–10 y, there is no accepted recommendation (12). We used a BMI less than the 5th centile according to the sex- and race-specific tables of Must et al (13, 14) to define wasting. The relation of iron deficiency and anemia to stunting and wasting was examined separately for children younger than and older than 10 y, to separate prepubertal and pubertal growth patterns.

Anemia was defined as a hemoglobin value < 110 g/L. This anemia cutoff value is lower than the WHO–recommended cutoff for this age group, but a race-specific anemia criterion (10 g/L lower for blacks) optimizes the screening performance of this indicator to detect iron deficiency (15). Distributions of EP and SF were skewed to high values so geometric means are presented. Iron deficiency anemia was defined as anemia with a SF concentration < 18 μg/L or an EP concentration > 90

μmol/mol heme. Severe anemia was defined as a hemoglobin value < 70 g/L.

The categorizations of age and parasitic infections in **Tables 1–3** were based on relations observed in exploratory analyses of those variables and iron-status indicators. For example, malaria parasitemia was considered as a dichotomous variable because there was no evidence of an empirical relation of parasite density to the iron-status indicators. Because hookworm and *T. trichiura* egg counts were linearly related to iron-status indicators, increasing categories of 2000 epg were used in subsequent analyses (Table 2).

Means of continuous variables were compared by using Student's *t* test, proportions were compared by using the chi-square test, and linear trends in mean values and proportions were tested by linear regression. Multivariate-linear-regression models of hemoglobin, EP, and SF were built in a forward, stepwise fashion. Variables were entered into the model in the order of their strength of association in the bivariate tables, and those that did not remain significant at a probability level of 5% were not retained. Interaction terms were considered for age, but none were retained using a probability level of 15% as criterion for significance.

To assess which risk factors were associated with the greatest proportion of the anemia in this population, we estimated the attributable fraction of anemia for each risk factor. This is analogous to attributable risk, except that anemia prevalence was used in place of incidence. The equation for attributable risk and its variance were taken from Kahn (16).

Data were entered by using EpiInfo software and were managed and analyzed by using SYSTAT statistical software (SYSTAT Inc, Evanston, IL).

RESULTS

The sample comprised approximately equal numbers of boys and girls, mostly between the ages of 7 and 13 y (**Table 4**). Parasitic infections were extremely prevalent. The prevalences of hookworm, *T. trichiura*, and *A. lumbricoides* infections were 94%, 96%, and 72%, respectively, and 99% of children were infected with at least one of these helminths. Of the 3361 children with complete parasitologic data, only one child was negative for all three helminths, malaria, and hematuria. Growth retardation was also highly prevalent.

The iron status of the children was very poor (Table 1). Overall, 62.3% of children were anemic and 51.5% had iron deficiency anemia by our definition. The overall prevalence of iron-deficient erythropoiesis (EP > 90 μmol/mol heme) was 59.7%, and the prevalence of exhausted iron stores (SF < 12 μg/L) was 41.3%.

By all indicators, the iron status of boys was slightly worse than that of girls (Table 1). The EP and SF values supported the fact that iron status was best in 7–10-y olds, slightly worse in children 11 y and older, and notably the worst in the small group of children younger than 7 y. In contrast, hemoglobin concentration and anemia prevalence improved with age, with no trend toward worsening values in children 11 y and older.

Hemoglobin concentration and anemia prevalence were significantly worse in stunted children, in both children younger than and older than 10 y. Overall, the hemoglobin concentration ($\bar{x} \pm$ SD) was 102 ± 16 g/L in children with a height-for-age Z score < −2, and 105 ± 16 g/L in children with Z scores ≥ −2 ($P < 0.001$). The prevalences of anemia and iron deficiency anemia were also higher in stunted children than in nonstunted children (66.3% compared with 58.6% for anemia, 54.7% compared with 48.6% for iron deficiency anemia; $P < 0.001$ for both comparisons). However, iron status as assessed by EP and SF was not worse in stunted children. Wasting was not associated with iron status by any indicator.

By far the most dramatic relation to iron status was that of hookworm infection (Table 2). This intensity-dependent relation was strongly apparent in every indicator. For example, hemoglobin concentration decreased by ≈50 g/L for each 2000 hookworm epg. Particularly noteworthy is the relation of hookworm infection to severe anemia. Among children with ≥ 6000

TABLE 1
Iron-status indicators by sex and age

	Hemoglobin	Protoporphyrin	Serum ferritin	Anemia[1]	Severe anemia[2]	Iron deficiency anemia[3]
	g/L	*μmol/mol heme*	*μg/L*	%	%	%
Sex						
Boys (*n* = 1864)	103 ± 16[4,5]	99 (56, 174)[5,6]	13.4 (6.9, 25.9)[5,6]	65.6[5]	4.1[5]	55.7[5]
Girls (*n* = 1731)	106 ± 15	95 (55, 163)	14.8 (7.5, 29.0)	58.8	2.8	47.2
Age (y)						
< 7 (*n* = 46)	92 ± 16[7]	128 (75, 217)[7]	11.7 (6.1, 22.5)[7]	73.9	19.6[7]	67.4
7.0–8.9 (*n* = 734)	103 ± 16	94 (54, 161)	14.5 (7.4, 28.4)	63.0	4.4	50.8
9.0–10.9 (*n* = 1585)	104 ± 16	94 (54, 164)	14.4 (7.3, 28.3)	62.8	2.6	50.6
11.0–12.9 (*n* = 935)	104 ± 16	100 (58, 174)[7]	13.7 (7.0, 26.9)[7]	61.7	3.5	53.3
≥ 13 (*n* = 295)	105 ± 16	104 (61, 178)[7]	12.7 (6.6, 24.6)[7]	57.6	2.7	50.2
All children (*n* = 3595)	104 ± 16	97 (56, 166)	14.0 (7.2, 27.3)	62.3	3.5	51.5

[1] Hemoglobin < 110 g/L.
[2] Hemoglobin < 70 g/L.
[3] Anemia with protoporphyrin > 90 μmol/mol heme or ferritin < 18 μg/L.
[4] $\bar{x} \pm$ SD.
[5] Significantly different from girls, $P < 0.05$.
[6] Geometric mean (−1 SD, +1 SD).
[7] Significantly different from children aged 7.0–10.9 y, $P < 0.05$ (chi-square test).

TABLE 2
Iron-status indicators by parasitic infections

Parasitic infection	Hemoglobin	Protoporphyrin	Serum ferritin	Anemia[1]	Severe anemia[2]	Iron deficiency anemia[3]
	g/L	*μmol/mol heme*	*μg/L*	*%*	*%*	*%*
Malaria parasitemia						
Negative (n = 1406)	105 ± 16[4,5]	93 (55, 156)[5,6]	14.1 (7.2, 27.9)[5]	58.6[5]	3.0[5]	46.3[5]
Positive (n = 2164)	103 ± 16	99 (57, 174)	13.9 (7.1, 27.2)	64.8	3.8	55.1
Microhematuria[7]						
Negative (n = 2469)	104 ± 16	97 (56, 169)	14.0 (7.3, 27.2)	62.9	3.4	52.4
Positive (n = 1096)	104 ± 16	96 (56, 163)	14.1 (7.3, 27.2)	60.9	3.5	49.5
Hookworms						
0 epg (n = 215)	110 ± 13[8]	84 (49, 142)[8]	18.3 (9.6, 34.6)[8]	46.5[8]	0.9[8]	33.5[8]
1–1999 epg (n = 2562)	105 ± 15	93 (56, 155)	14.8 (7.9, 27.9)	60.0	2.5	48.7
2000–3999 epg (n = 456)	100 ± 17	107 (63, 183)	11.5 (6.0, 21.7)	73.7	5.9	64.7
4000–5999 epg (n = 128)	96 ± 19	127 (75, 216)	9.9 (5.2, 18.7)	79.7	10.2	71.1
≥ 6000 epg (n = 67)	93 ± 23	152 (90, 259)	8.1 (4.3, 15.4)	77.6	14.9	74.6
Trichuris trichiura						
0 epg (n = 137)	106 ± 16	97 (56, 166)	15.4 (8.0, 29.8)[8]	60.6	2.2	52.6
1–1999 epg (n = 2642)	104 ± 16	96 (54, 170)	14.3 (7.2, 28.5)	61.7	3.4	50.4
2000–3999 epg (n = 428)	104 ± 16	97 (56, 166)	13.4 (6.9, 25.8)	63.3	3.5	54.4
4000–5999 epg (n = 124)	103 ± 16	99 (58, 169)	13.1 (6.8, 25.3)	66.9	4.0	55.7
≥ 6000 epg (n = 96)	103 ± 16	98 (58, 166)	12.1 (6.2, 23.4)	62.5	4.2	50.0
Ascaris lumbricoides						
Negative (n = 961)	106 ± 16[5]	96 (56, 162)	14.6 (7.4, 28.6)	56.5[5]	3.1	46.3[5]
Positive (n = 2466)	104 ± 16	97 (56, 167)	14.0 (7.2, 27.2)	64.2	3.5	53.1

[1] Hemoglobin < 110 g/L.
[2] Hemoglobin < 70 g/L.
[3] Anemia with protoporphyrin > 90 μmol/mol heme or ferritin < 18 μg/L.
[4] $\bar{x}$ ± SD.
[5] Significantly different from positive, $P < 0.05$.
[6] Geometric mean (−1 SD, +1 SD).
[7] An indicator of urinary schistosomiasis.
[8] Significant linear trend with increasing intensity of infection, $P < 0.05$.

hookworm epg, the prevalence of severe anemia was nearly 15%. Children with circulating malaria parasites had hemoglobin and EP concentrations slightly but significantly worse than children without parasitemia; however, SF concentrations were not different. *T. trichuris* infection was associated with lower SF, and this relation was intensity-dependent, but no other indicator of iron status was related to *T. trichuris*. *A. lumbricoides* infection was associated with lower hemoglobin concentrations, but not with EP or SF. Hematuria was not related to iron status.

When demographic, anthropometric, and parasitologic variables were considered jointly in multivariate models, hookworm remained the strongest explanatory variable for hemoglobin, EP, and SF. For SF, the only variables retained in the multivariate model were hookworm infection and age > 11 y. For EP, hookworm infection, age < 7 y, and stunting were retained. The final multivariate model for hemoglobin concentration was more complex. Hookworm infection, sex, age < 7 y, *A. lumbricoides* infection, malaria parasitemia, and stunting were significant and all appeared to have independent associations with hemoglobin because the regression coefficients from the multivariate model were practically identical to the bivariate differences shown in Tables 1 and 2. Wasting and age > 11 y were not retained in the final hemoglobin model.

To assess the importance of parasitic infections as causes of anemia in this population, we estimated the attributable fraction of the three forms of anemia associated with the parasites (Table 3). We also calculated an attributable fraction for stunting because it may be considered an indicator of a chronically poor diet. Sex and age, although significantly associated with anemia prevalence, were not considered because they are not preventable. About one-fourth of all anemia, one-third of iron deficiency anemia, and three-fourths of severe anemia were attributable to hookworm infection in this population of schoolchildren. Eleven percent or less of anemia and iron deficiency anemia were attributable to malaria, *A. lumbricoides* infection, or stunting.

DISCUSSION

Evidence of iron deficiency anemia

Our findings confirm that iron deficiency anemia is an urgent problem among schoolchildren in coastal East Africa. In the context of sub-Saharan Africa, Pemba Island is underdeveloped with regard to health and nutrition status, but it is not unique in the prevalence of iron deficiency or parasitic infections. Similarly, poor iron status of schoolchildren has been reported in other studies from both west (17) and east (18, 19) Africa. Given the relative feasibility of addressing parasitic infections and iron deficiency through school health programs, public health actions should be carefully considered.

The pattern of EP and SF concentrations by age remained significant in multivariate models and are biologically plausi-

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TABLE 3
Attributable fractions of anemia associated with preventable risk factors[1]

Form of anemia and risk factor	Prevalence of risk factor	Prevalence ratio[2]	Attributable fraction[2]
	%		%
All anemia			
Hookworm infection	94	1.36 (1.17, 1.57)	25 (15, 35)
Ascaris infection	72	1.14 (1.07, 1.21)	9 (5, 13)
Malarial infection	61	1.11 (1.05, 1.17)	6 (3, 9)
Stunting	48	1.13 (1.08, 1.19)	6 (3, 8)
Iron deficiency anemia			
Hookworm infection	94	1.56 (1.29, 1.89)	35 (23, 47)
Ascaris infection	72	1.15 (1.06, 1.24)	10 (5, 15)
Malarial infection	61	1.19 (1.11, 1.27)	10 (7, 14)
Stunting	48	1.15 (1.06, 1.20)	6 (3, 9)
Severe anemia			
Hookworm infection	94	3.81 (0.95, 15.33)	73 (110, 35)

[1] All anemia defined as a hemoglobin concentration < 110 g/L; iron-deficiency anemia defined as anemia with protoporphyrin > 90 μmol/mol heme or ferritin <18 μg/L; severe anemia defined as a hemoglobin concentration < 70 g/L.
[2] 95% CIs associated with risk factor in parentheses.

TABLE 4
Characteristics of the study sample[1]

	Frequency
	%
Age (y)	
< 7	1.3
7.0–8.9	20.4
9.0–10.9	44.1
11.0–12.9	26.0
≥ 13	8.2
Sex (boys)	51.8
Malaria parasitemia	60.6
Hookworm infection	
Negative (0 epg)	6.3
Light (1–1999 epg)	55.5
Moderate (2000–4999 epg)	34.9
Heavy (≥ 5000 epg)	3.3
Trichuris trichiura infection	
Negative (0 epg)	4.0
Light (1–1999 epg)	77.1
Moderate (2000–9999 epg)	17.9
Heavy (≥ 10 000 epg)	1.0
Ascaris lumbricoides infection	
Negative (0 epg)	28.0
Light (1–9999 epg)	61.7
Moderate (10 000–49 999 epg)	10.3
Heavy (≥ 50 000 epg)	0.1
Microhematuria[2]	
Negative	69.2
Positive	30.8
Stunted[3]	47.6
Wasted[4]	35.3

[1] epg, eggs per gram feces.
[2] An indicator of urinary schistosomiasis.
[3] Height-for-age Z score < −2.
[4] Body mass index below the 5th centile of reference population.

ble. The youngest children (< 7 y) would have most recently experienced the high iron demands of early childhood, whereas children aged ≥ 11 y may have been experiencing the pubertal growth spurt. Boys had EP and SF values indicative of greater iron deficiency compared with girls, but this sex difference did not remain significant in multivariate analyses. Because boys tended to have more intense hookworm infections than girls, the sex difference disappeared when we controlled for hookworm infection intensity. Other studies of iron status of schoolchildren have also found no important sex differences in SF until the later teenage years (20–22).

Evidence for non-iron deficiency anemia

Anemia can stem from a variety of causes, and it is apparent in this population that iron deficiency does not account for all the anemia observed. Whereas EP rose and SF declined at ages > 11 y, hemoglobin did not change significantly. Also, stunting was associated with a lower hemoglobin concentration but was not associated with EP or SF. Lastly, *A. lumbricoides* infection and malarial parasitemia remained significant in the multivariate model for hemoglobin, but were not retained in the models of EP and SF after hookworms were controlled for. These findings suggest that factors other than poor iron status contribute to the low hemoglobin concentrations in this population. It is possible that one or more other nutrient deficiencies link stunting, ascariasis, or malaria to erythropoiesis.

The negative association of hemoglobin to *A. lumbricoides* (23) and malaria (24–26) infections has been reported by others as well. Hemoglobin may be lowered in a nonspecific way during infection—the so-called anemia of chronic disease (27–29). However, it is unlikely that the acute-phase response to infection with malaria or *A. lumbricoides* would influence hemoglobin more than EP or SF. As we described in greater detail elsewhere (7), there was no decreasing trend in hemoglobin concentration as malarial parasitemia increased in this population. We hypothesize that this is because Zanzibari children are exposed to *P. falciparum* repeatedly in their preschool years, and by school age they have developed significant resistance to this infection (7). The difference in hemoglobin concentration between children with and without these infections was significant but small (20 g/L for both malaria and ascariasis).

Risk factors for iron deficiency anemia

The most impressive finding in this study was the contribution of hookworms to the burden of iron deficiency and anemia. The strength of the association we observed is explained by the range of hookworm infection intensities in the population, and the lack of storage iron that would buffer the effect of hookworm-related blood loss on iron status (30). Our data confirm the findings of Stephenson et al (18, 31) in coastal Kenya, in which the hemoglobin deficit associated with hookworm infection was greater than that of either schistosomiasis or malaria among schoolchildren.

Although we did not assess dietary iron intake, it is also clear from our data that dietary iron intakes were seriously inadequate. Even among children with no hookworm infection, the prevalence of anemia was 46.5%, and the average values for hemoglobin, EP, and SF only bordered on adequacy. Although anemia was prevalent in children without hookworm, severe anemia was rare (< 1%). If one imagines a normal hemoglobin distribution, it appears that dietary deficiency shifts the distribution significantly to the left, driving many children into a

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state of mild iron deficiency anemia. The addition of hookworms pulls the center of the distribution even farther left, and dramatically pulls down the left tail.

The intensity-dependent association between *T. trichiura* infection and SF concentration is likely explained by their common association with hookworm, because *T. trichiura* infection did not remain significant in the multivariate model of SF. The presence and intensity of *T. trichiura* infection was significantly associated with hookworm-infection intensity, despite the different routes of transmission of these two helminths. One clinical study found intestinal blood loss associated with *T. trichiura* infection (32), but another did not (33). We studied intestinal iron loss in this population and found no association between fecal hemoglobin concentration and *T. trichiura* infection after hookworm infection was controlled for (34). Thus, we conclude that the cross-sectional association between iron-status indicators and *T. trichiura* infection observed by us and others (35) was probably not caused by *T. trichiura*–associated blood loss.

Other investigators have found a strong association between urinary schistosomiasis and iron status in sub-Saharan Africa (18, 31, 36). The lack of association in our study may be due to several reasons. Most probably, it is because a highly effective test-and-treat campaign for schistosomiasis was conducted on Pemba Island from 1988 to 1992 (37). Thus, children with a positive indication of schistosomiasis in our study may have had the disease for only a short duration. Also, hookworm infection was significantly less common in children with schistosomiasis in this sample. Finally, hematuria is an excellent field tool but an imperfect diagnostic indicator of schistosomiasis (11), and misclassification would attenuate a true association. Use of quantitative egg counts in urine would be a more powerful way to examine this relation.

Implications for control of iron deficiency anemia

The most important application of these findings is to guide development of appropriate interventions to control iron deficiency anemia in African schoolchildren. Our results provide a strong basis for hookworm control as a strategy to control iron deficiency in this population group. If hookworms could be eradicated, anemia might be reduced by as much as 25%, iron deficiency anemia by 35%, and severe anemia by 73%. Because of the intensity-dependent relation of hookworm infection to intestinal iron loss and iron status, reducing hookworm burdens (rather than eradicating infection) is the most rational short- to medium-term goal (38). Anthelminthic drugs are safe and inexpensive (≈US$0.025-0.20 per treatment), and periodic delivery of them through the school system is a highly feasible public health intervention (39). Our findings indicate that hookworm control will have the greatest effect on the lower tail of the hemoglobin distribution, ie, preventing relatively severe anemia.

Stopping the leakage of iron from children's bodies will not replenish their stores however, and thus hookworm control alone will not solve the problem. Additional intake of iron will be needed. In Zanzibar and many other parts of sub-Saharan Africa, iron fortification of foods is not yet feasible. School-based iron supplementation could be an opportunity to make up the iron deficit that many children bring with them from the preschool years, and to build stores to carry them into adulthood. Applied research is needed to find the most effective dosing regimens to achieve these goals.

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IODINE DEFICIENCY

Infant Survival Is Improved by Oral Iodine Supplementation[1,2]

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ABSTRACT Although reports suggest that infant mortality is increased during iodine deficiency, the effect of iodine supplementation on infant mortality is unknown. A double-masked, randomized, placebo-controlled, clinical trial of oral iodized oil was conducted in Subang, West Java, Indonesia to evaluate the effect of iodine supplementation on infant mortality. Infants were allocated to receive placebo or oral iodized oil (100 mg) at about 6 wk of age and were followed to 6 mo of age. Six hundred seventeen infants were enrolled in the study. Infant survival was apparently improved, as indicated by a 72% reduction in the risk of death during the first 2 mo of follow-up ($P < 0.05$) and a delay in the mean time to death among infants who died in the iodized oil group compared with infants who died in the placebo group (48 days vs. 17.5 d, $P = 0.06$). Other infant characteristics associated with reduced risk of death included weight-for-age at base line, consumption of solid foods, female gender and recent history of maternal iodine supplementation. Oral iodized oil supplementation had a stronger effect on the mortality of males compared with females. This study suggests that oral iodized oil supplementation of infants may reduce infant mortality in populations at risk for iodine deficiency. J. Nutr. 127: 574–578, 1997.

KEY WORDS: • *infants* • *mortality* • *iodine deficiency* • *goiter* • *cretinism*

Approximately 1.6 billion people worldwide may consume inadequate daily amounts of iodine (UNICEF 1995) and are at risk for iodine deficiency disorders (IDD)[4] (Hetzel and Pandav 1994), which include enlargement of the thyroid (goiter) and a wide spectrum of mental, psychomotor and growth abnormalities (Delange 1994). Currently, there are an estimated 655 million cases of endemic goiter and 26 million cases of preventable mental deficiency, including 5.7 million cases of cretinism worldwide (Hetzel and Pandav 1994, WHO 1991b). Seventy-five percent of people with goiter live in developing countries (Gaitan et al. 1991). In areas with a high prevalence of IDD, maternal and fetal iodine deficiency has been associated with increased rates of stillbirth, abortion, congenital anomaly, and infant mortality (Pharoah et al. 1976, Thilly et al. 1980). Iodine supplementation of women before conception or during pregnancy seems to reduce abortions, stillbirths and infant mortality (Chaouki and Benmiloud 1994, Fierro-Benitez et al. 1988, Glinoer et al. 1995, Hetzel 1983, Pharoah and Connolly 1987, Pharoah et al. 1971 and 1972, Potter et al. 1979). Although these studies suggest that iodized oil supplementation of infants might improve their survival, this has not been shown definitively.

Oral iodized oil supplementation might influence infant survival through its effect on thyroid status and immunity. Thyroid hormones exert a powerful modulating effect on the immune system (Fabris 1973), including effects on B cell differentiation (Paavonen 1982), antibody responses (Keast and Ayre 1980), lymphoproliferation to mitogen (Keast and Taylor 1982) and T cell subsets (Ohashi and Itoh 1994). Although the relationship between thyroid disease and autoimmunity is well documented (Mooij and Drexhage 1992), less is known regarding IDD and immune function in humans. Thyroid status and vitamin A status may also potentially interact to modulate immune responses. Both an active metabolite of vitamin A, 9-*cis* retinoic acid, and thyroid hormone can bind to retinoid*X receptor (Leng et al. 1994) and control gene transcription.

SUBJECTS AND METHODS

The study population consisted of mothers and their infants from 28 villages in an area of mild-to-severe IDD in Subang, West Java, Indonesia. Eligible infants were 6–10 wk old, clinically euthyroid, and born in the study villages. The time of supplementation of 6–10 wk of age was based upon the possible programmatic integration of oral iodized oil supplementation with the first visit of the Expanded Programme on Immunization (EPI) (Bruning et al. 1993, WHO 1987, 1990 and 1991a) at which time infants received oral poliovirus and diphtheria-pertussis-tetanus vaccines. The study design was a randomized, double-masked, placebo-controlled, clinical trial. Infants were allocated by random number table in blocks of 10 to receive oral

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[4] Abbreviations used: EPI, Expanded Programme on Immunization; IDD, iodine deficiency disorders; WAZ, weight-for-age Z-score.

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iodized oil or placebo, which was given at the first EPI visit. Upon enrollment, infants were assigned sequential identification numbers. An envelope labeled with the identification number contained identically appearing capsules of either oral iodized oil in poppyseed oil (100 mg) or poppyseed oil placebo (Lipiodol, Laboratoire Guerbet, Aulnay-sous-bois, France). The code was unknown to the investigators and study team until after the completion of the study. The capsules were administered immediately after the first dose of oral polio vaccine. Infants in the clinical trial then completed the immunization schedule of the EPI with visits at 10 and 14 wk of age, and a final follow-up evaluation at 6 mo of age.

A 7-d morbidity history was obtained at each visit. Height, weight, mid-upper arm circumference and head circumference were assessed at the first, third and final visits using standard procedures. At each visit, mothers were asked whether their infants were being breast-fed and/or given solid food or other liquids besides breastmilk, how many times a day they were breast-fed and/or fed other foods, and the list of other foods given. Community health workers visited the homes of participants on a monthly basis and monitored the study population for deaths, including those among infants who dropped out of the study but remained in their villages. Probable causes of death were determined by the pediatrician after interviewing relatives of the deceased infant using verbal autopsy techniques adapted from other pediatric studies (Dowell et al. 1993, Kalter et al. 1990).

Base-line and follow-up comparisons between treatment groups were made using Student's *t* test for comparison of two means and Fisher's exact test for comparison between proportions, with statistical significance at a two-tailed *P* value of 0.05 or less. Absolute and gender-stratified relative risk, mortality rates and Kaplan-Meier survival curves were calculated and compared between allocation groups (Armitage and Berry 1987). Adjusted survival analyses were conducted using Cox regression models (Kleinbaum et al. 1982). Written informed consent was obtained from parents or guardians. The study was approved by the institutional review board at the Johns Hopkins School of Medicine and by the ethical committees of the Hasan Sadikin Hospital, University Pajajaran and the Ministry of Health, Government of Indonesia.

RESULTS

From June to October 1994, 617 infants were recruited to participate in the study. Of the 617 participants, 307 (49.8%) infants received oral iodized oil and 310 (50.2%) received placebo; 311 (50.4%) infants were males and 306 (49.6%) were females. The treatment groups were similar by maternal goiter status, maternal age, infant gender, socioeconomic status, use of iodized salt and all other characteristics at base line, some of which are displayed on **Table 1.** Five hundred seven infants completed follow-up to 6 mo, 255 of 307 in the iodized oil group (83%), and 252 of 310 (81%) in the placebo group. There was no differential loss to follow-up by treatment group or gender. Eighty-eight infants dropped out of the study before 6 mo of age. Their base-line characteristics were similar to those who remained in the study (data not shown). A total of 22 infants died, 15 (68.2%) in the placebo group and 7 (31.8%) in the iodized oil group. Of those who died, 14 (63.6%) were male and 8 were female (36.4%).

Infants who died before 6 mo of age were significantly lighter and had smaller head and arm circumferences at study entry than the infants who survived to 6 mo of age. Their mean weight-for-age was also significantly lower, indicating that they were malnourished at base line in comparison with the surviving infants (Table 2). None of the mothers whose infants died had a history of iodized oil supplementation in comparison with 86 (17%) of the 507 mothers whose infants survived to 6 mo of age ($P = 0.034$). Supplemented mothers had received oral iodized oil on average 120 d before the beginning of the study. No other base-line differences were observed between those who survived to 6 mo of age and those who did not. The percentage of mothers who had been supplemented in the past and the time elapsed between maternal and infant supplementation were similar in the iodized and placebo groups.

Deaths in the iodized oil group occurred on average 48 d ($\pm$ 13.1) after the intervention, whereas in the placebo group, deaths occurred on average 17.5 d ($\pm$ 4.0) after the initial visit ($P = 0.06$). There were 13 deaths (65%) due to pneumonia or bronchopneumonia, 1 death (5%) due to meningoencephalitis, 1 death (5%) due to asphyxia, and 2 deaths with unspecified diagnoses (allergic reaction and shock of undetermined nature). Five deaths were not investigated through verbal autopsies. The unadjusted relative risk of death in the iodized oil group in comparison with the placebo group during the first 1, 2 and 4 mo of follow-up were 0.19 (95%CI 0.04–0.85, $P = 0.021$); 0.28 (95%CI 0.09–0.82, $P = 0.018$); and 0.48 (95%CI 0.20–1.15, $P = 0.126$), respectively. **Figure 1** shows the Kaplan-Meier survival curves according to treatment group. At the end of 4 mo of follow-up, the overall mortality experience in the iodized oil group was still considerably lower than that of the placebo group, corresponding to a 52% reduction in mortality in the supplemented group. The final mortality difference at 6 mo of age, however, failed to reach statistical significance, perhaps due to the small sample size studied. The power to detect a difference such as the one observed at 6 mo of age was approximately 32%.

Multivariable Cox regression analysis was used to measure the effect of other independent variables on survival, to control for potential confounding and to test for interactions. Variables analyzed included gender, infant's diet at base line (breastfeeding and/or introduction of solid foods), diarrhea or acute respiratory infection (cough, difficult breathing, or fast breathing) at base line, history of maternal iodized oil supplementation, anthropometry at base line, and interaction among treatment, gender and other variables showing association with survival. Iodine supplementation, consumption of solid foods or liquids at base line, female gender, weight, weight-for-age Z-score (WAZ), and head and arm circumference were independently associated with improved survival. WAZ was selected for the final model on the basis of the magnitude and statistical significance of the beneficial effect on survival and multicolinearity between anthropometric parameters. **Table 3** presents the results of the multivariable Cox survival analysis with the effect of iodine supplementation adjusted for gender, WAZ and consumption of solid foods at base line. Treatment effect was not confounded and did not interact with the effect of solid foods and anthropometry at base line. Sex-stratified analysis of treatment effect indicated that male survival at 6 mo of age in the iodized oil group was significantly better than that of male infants in the placebo group (22.9 per 1000 vs. 84.6 per 1000, $P = 0.03$). The *P*-value for the interaction between treatment and sex in the Cox regression model was 0.15. Because the difference in mortality occurred soon after enrollment, there were limited data regarding time-dependent variables such as anthropometry and diet for the infants who died; therefore, the Cox regression analysis was limited to base-line variables only.

Among 326 infants whose base-line diets included foods or liquids, 164 (50.0%), 126 (38.4%), 35 (11.3%) and 1 (0.3%) were fed solid foods or liquids 1, 2, 3 and 4 times/d, respectively. Other liquids consisted of water, tea with sugar or honey, or fruit juice. Solid or mushy foods included premasticated rice (bubur), mashed bananas, biscuits, industrialized infant food (e.g., Promina and Farley) and infant formulas (e.g., Lactogen, SNN and SGN). The proportion of infants breast-fed at base line was lower, but not significantly so, among infants who received supplements (95.7 vs. 97.9%, $P = 0.125$). Infants who received solid foods or liquids supplements at base line were slightly, but significantly older at study

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TABLE 1

Selected base-line characteristics by allocation group

	Placebo n = 310 n (%)	Iodized oil n = 307 n (%)	P-value
Maternal characteristics			
Goiter grade[1]			
00	275 (89.6)	267 (87.6)	0.54
1A	20 (6.5)	26 (8.5)	
1B	7 (2.3)	8 (2.6)	
02	3 (1.0)	4 (1.3)	
03	2 (0.6)	0 (0.0)	
Previous maternal iodine supplementation	52 (16.8)	47 (15.3)	0.66
Use of vitamin and mineral supplements during pregnancy	259 (84.6)	261 (85.6)	0.82
Mean age,[1,2] *y*	25.4 ± 0.32	25.7 ± 0.32	0.61
Pregnancies, *n*	2.3 ± 0.09	2.3 ± 0.08	0.69
Deliveries, *n*	2.2 ± 0.09	2.2 ± 0.08	0.76
Live births, *n*	2.2 ± 0.09	2.2 ± 0.08	0.69
Children alive, *n*	2.1 ± 0.08	2.1 ± 0.07	0.98
Maternal education, *y*	7.1 ± 0.15	7.4 ± 0.16	0.32
Maternal occupation: housewife	272 (94.1)	266 (90.5)	0.32
Infant gender, age at base line, and delivery history			
Gender			
Male	157 (50.6)	154 (50.2)	0.90
Female	153 (49.3)	153 (49.8)	
Place of delivery			
Home	269 (86.8)	272 (89.2)	0.66
Midwife's house	16 (5.1)	11 (3.6)	
Hospital	17 (5.5)	17 (5.6)	
Clinic	8 (2.6)	5 (1.6)	
Delivery assisted by			
Traditional birth attendant	235 (76.1)	237 (77.5)	0.38
Midwife	65 (21.0)	65 (21.2)	
Doctor	9 (2.9)	4 (1.3)	
Mean birth weight, *kg*[1]	3.20 ± 0.03	3.20 ± 0.03	0.74
Mean age, *mo*	1.82 ± 0.02	1.80 ± 0.02	0.54

[1] Data from placebo group (n = 307), iodized oil group (n = 305).
[2] Mean ± SEM.

entry than infants who did not receive supplements (1.9 vs. 1.8 mo, $P < 0.0001$). They were also heavier and taller (4.9 vs. 4.7 kg, $P = 0.0041$; 56.2 vs. 55.5 cm, $P = 0.0003$), had slightly greater head and arm circumference (38.0 vs. 37.7 cm, $P = 0.002$; and 12.5 vs. 12.4 cm, $P = 0.05$), but similar age adjusted Z-scores (WAZ 0.03 vs. −0.03, $P = 0.371$; height-for-age Z-score −0.34 vs. −0.45, $P = 0.109$; weight-for-height Z-score 0.37 vs. 0.39, $P = 0.622$). Infants who did not receive solid foods were more likely to have a better socioeconomic status as indicated, for instance, by the significantly higher percentage living in houses with electricity and higher mean years of parental schooling (data not shown). The effect of supplemental foods/liquids on survival did not change when controlled for age, socioeconomic status, iodine supplementation, WAZ or gender.

TABLE 2

Birth weight and base-line age and anthropometry by infant vital status

	Dead n = 22	Alive n = 507	P-value
Birthweight,[1,2] *kg*	3.0 ± 0.11	3.2 ± 0.02	0.10
Age at base line, *mo*	1.7 ± 0.07	1.8 ± 0.01	0.32
Weight, *kg*	4.5 ± 0.14	4.8 ± 0.03	0.03
Length, *cm*	55.0 ± 0.49	55.8 ± 0.11	0.12
Head circumference, *cm*	37.2 ± 0.24	37.9 ± 0.06	0.01
Arm circumference, *cm*	11.9 ± 0.21	12.5 ± 0.05	0.02
Weight-for-age Z-score	−0.43 ± 0.17	0.03 ± 0.04	0.01
Height-for-age Z-score	−0.70 ± 0.20	−0.38 ± 0.04	0.09
Weight-for-height Z-score	0.16 ± 0.17	0.40 ± 0.03	0.11

[1] Mean ± SEM.
[2] For birthweights, those who died (n = 17), those alive (n = 331).

DISCUSSION

This study suggests that iodine (100 mg) given at 6 wk of age may reduce the risk of death among infants in a geographi-

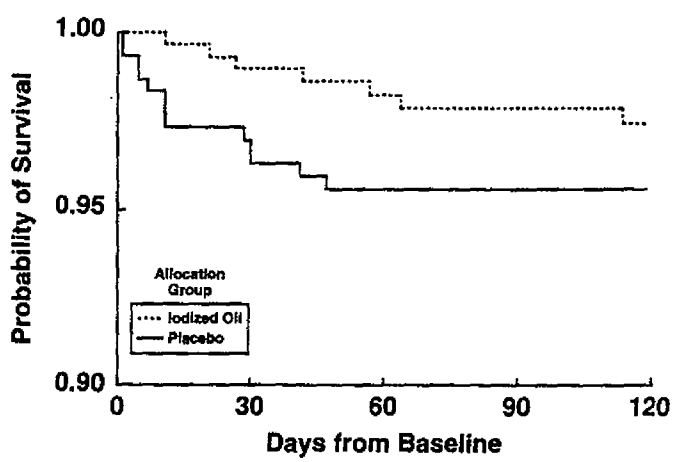

FIGURE 1 Survival of infants in the oral iodized oil and placebo groups from time of supplementation through 120 d of follow-up. Age at base line was 6 wk.

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TABLE 3

Relative risk (RR) of death and 95% confidence interval (CI) at 1, 2 and 4 mo after study entry according to iodine supplementation adjusted for gender, weight-for-age Z-score and consumption of solid foods at base line

Variable	1 mo	2 mo	4 mo
	RR (95% CI; *P*)	RR (95% CI; *P*)	RR (95% CI; *P*)
Iodized oil vs. control	0.20 (0.04–0.91; 0.04)	0.30 (0.10–0.90; 0.03)	0.52 (0.21–1.28; 0.15)
Solid food vs. no solid food	0.41 (0.12–1.35; 0.14)	0.41 (0.15–1.09; 0.08)	0.41 (0.17–1.01; 0.05)
Female vs. male	0.54 (0.18–1.68; 0.29)	0.39 (0.15–1.04; 0.06)	0.49 (0.20–1.18; 0.11)
Weight-for-age Z-score at base line	0.58 (0.30–1.13; 0.11)	0.53 (0.30–0.92; 0.02)	0.55 (0.33–0.91; 0.02)

cal area of iodine deficiency. The absence of deaths among infants whose mothers received iodine supplementation in the recent past was another finding in this study that also supports the potentially beneficial role of iodine in reducing mortality. To our knowledge, this is the first study to demonstrate that infant oral iodine supplementation can reduce infant mortality. This finding is corroborated by previous studies that suggest that infant survival is improved among infants born to women whose iodine deficiency was corrected before or during pregnancy. In Algeria, the rates of abortion, stillbirth and prematurity were significantly lower among women treated with oral iodized oil 1–3 mo before conception or during pregnancy than among untreated women (Chaouki and Benmiloud 1994). Infant mortality was lower among infants born to women who received intramuscular iodized oil supplementation at around 28 wk of pregnancy compared with infants born to controls in a clinical trial from Zaire (Thilly et al. 1980). Studies in Papua New Guinea indicated an inverse relationship between levels of maternal thyroxine during pregnancy and death rates among the offspring (Pharoah et al. 1976), and an improved long-term survival among children born to women whose iodine deficiencies were corrected during or shortly before pregnancy (Pharoah and Connolly 1987). A possibly related finding is a recent report of significantly reduced mortality among young sheep in villages receiving iodine in irrigation water compared with control villages in China (Cao et al. 1994).

This study suggests that there may be a possible differential effect of treatment according to gender. Nutritional and immunological differences between boys and girls may influence response to infections and survival. For example, boys are more susceptible to vitamin A deficiency than girls, which is a consistent observation in different countries (Sommer 1982). Differences in immunologic status (Leon et al. 1993), responses to vaccination (Garenne et al. 1991) and micronutrient supplementation (Sazawal et al. 1996) are known between boys and girls. It is unknown whether boys are at higher risk of iodine deficiency or whether there is a gender difference in response to iodine supplementation. A treatment effect may not have been observed in girls because of their already high survival rate of 97%.

It is possible that infants receiving solid foods were exposed to commercial products which potentially provided them with additional sources of iodine. However, the study did not use systematic methodologies carefully designed for the evaluation of dietary practices or for adequate assessment of iodine content of foods. The finding of improved survival among infants having solid foods in the diet at study entry should be investigated further by means of a more precise dietary assessment. This study is limited in that biochemical indicators of iodine and thyroid status were not available. However, detailed anthropometric evaluation and clinical assessment of maternal goiter suggest that the observed differences in mortality were not due to base-line imbalances in the treatment group.

Traditionally, the main focus in the prevention of IDD has been to eliminate the ensuing mental impairment, cretinism and goiter. Ideally, adequate iodine levels should be made available from the beginning of fetal development in utero by adequate supplementation of women of childbearing age and pregnant women. The EPI may serve as an additional delivery system for providing supplementation to mothers and infants who missed earlier opportunities. The duration for which one 100-mg dose of iodine will correct iodine deficiency in a 6-wk-old infant is unknown; however, WHO (1991) estimates that a 240-mg dose of iodine administered once in the first year of life will correct iodine deficiency for up to 2 y. Infant iodine supplementation may be a potential intervention that could reduce infant mortality in iodine-deficient areas by 50%; further study is required to corroborate these findings with a larger sample size. Iodine supplementation may be an important strategy for child survival, and this study suggests that the infrastructure to deliver supplements could be provided by the Expanded Programme on Immunization.

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OTHER

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PARASITIC INFECTIONS IN PEMBA ISLAND SCHOOLCHILDREN
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PARASITIC INFECTIONS IN PEMBA ISLAND SCHOOLCHILDREN

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SUMMARY

Intestinal helminths, schistosomiasis and malaria have been recognised for decades to be major public health problems in Zanzibar, Tanzania. During the evaluation of the impact of the Zanzibar Helminth Control Programme, baseline parasitological data on 3,605 school children were collected in Pemba Island. Prevalence of intestinal helminth infections was 72%, 94% and 96% for *Ascaris lumbricoides, Trichuris trichiura* and hookworm, respectively. Thirty one percent of children tested positive for haematuria, a reliable indicator of urinary schistosomiasis in the study area. Malaria parasites were found in 61% of children. Hookworm infections and haematuria were more prevalent in boys. Sixty seven percent of the children were infected with all the three helminths, and 28% harboured double infection. No association was found between intestinal helminths and schistosomiasis or malaria. Children living in rural areas were more heavily infected with hookworms, schistosomiasis and malaria compared to children in towns. Results from this study provided relevant information for designing a "plan of action" for the integrated control of filariasis, intestinal helminths, malaria and schistosomiasis in Zanzibar.

INTRODUCTION

It is widely recognised that school children carry the heaviest burden of morbidity due to intestinal helminths and schistosomiasis infection(1). Apart from the morbidity associated with acute infections, the burden of chronic parasitic infections may affect physical fitness(2), cognitive performance(3), nutritional status and growth(4) and school attendance(5) of school age children. Acute and chronic malaria infections may affect school performance of children in endemic areas(6).

Parasitic infections have been recognised for decades to be major public health problems in Zanzibar, United Republic of Tanzania(7-9). Malaria is the major public health problem accounting for more than 30% of child mortality(10). The present strategy of malaria control is based on early diagnosis and prompt case treatment. Major constraints are the lack of an efficient referral system, increasing resistance of *Plasmodium falciparum* to chloroquine and shortage of second line antimalarial drugs. Schistosomiasis control was initiated in 1986 through a school-based approach and was integrated in 1994 within a National Plan of Action for the control of a schistosomiasis and intestinal helminthic infections. The strategy adopted was based on a combination of regular chemotherapy of school children with praziquantel and mebendazole administered as single doses and health education. Children were selected for treatment with praziquantel on the basis of the presence of haematuria(7), while mebendazole was given as targeted mass treatment due to the high prevalence and intensity of helminthic infections(8). An inexpensive and effective drug and health education delivery system was set up through the teachers with supervision from health staff of the helminth control programme.

In 1994 the Ministry of Health of Zanzibar, in collaboration with the World Health Organization (WHO) and The Johns Hopkins University, recognised the need to evaluate the impact of the Helminth Control Programme. A large scale longitudinal survey was planned to collect nutritional and parasitological data on growth, iron deficiency, vitamin A status, malaria, haematuria and prevalence and intensity of soil transmitted nematodes infection in school children. The baseline nutritional status data has been published elsewhere(11). The epidemiology and geographical distribution of parasitic infections, their association and the implication for integrated control are presented in this paper.

MATERIALS AND METHODS

The survey was conducted on the island of Pemba, Tanzania by the staff of the Pemba Helminth Control Programme. Important features of the island have been described in detail elsewhere(7).

The survey was carried out during April and May 1994 in 12 schools, randomly selected from the 72 schools on Pemba Island. Three schools were selected from each of the four

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districts. From these schools, children enrolled in 76 morning classes in standards one to four, were invited to participate in the study. Out of 3944 enrolled children, 3,605 participated in the survey.

In every school, demographic information, weight, height and stool, urine and blood samples were collected from each child. The sample collection rates were 100%, 99% and 97% for blood, urine and stool samples, respectively. Urine was tested with a reagent strip for semiquantitative values of haematuria (Hemastix, Ames-Miles Laboratories, Helkhart, IN). In this population, microhaematuria is considered to be diagnostic of urinary schistosomiasis (69% sensitivity and 88% specificity)(12). Children testing positive for visual haematuria were treated with praziquantel 40mg/kg body weight. Haemoglobin, erythrocyte protoporphyrin and serum ferritin were assessed as part of nutritional evaluation(11). Those with severe anaemia (Hb below 7 g/dl) received mebendazole 500 mg as a single dose and ferrous sulphate 25 mg tablets twice a day for one month. A thin and thick malaria smear was also made for each child. Children with fever were treated with a standard dose of pyrimethamine-sulphadoxine according to weight. All children were treated with mebendazole 500 mg as a single dose at the end of the trial.

In the laboratory, stool samples were processed by the Kato-Katz technique according to WHO recommendations(13) and examined for helminth eggs; samples containing more than 10,000 eggs were re-examined using a modified Stoll dilution technique(14). Thick and thin blood smears were stained with 3% Giemsa solution and malaria parasites were counted against leucocytes to calculate parasitaemia per microliter of blood(13), and identified by species. Quality control for malaria and intestinal helminths, was performed by two supervisors by re-examining 10% of the slides chosen at random. Agreement between readers was excellent for the presence of malaria parasitaemia (k=0.93) and parasite density were also highly reliable with an interclass correlation of 0.85. Interclass correlation between staff and quality control readings were 0.88 for *Ascaris lumbricoides* egg per gram (epg), 0.79 for *Trichuris trichiura* epg, and 0.89 for hookworm epg. Data were entered into a microcomputer using the Epi-Info package.

Statistical analysis: Intensity of intestinal helminth infections was measured by the eggs per gram of faeces (epg). The geometric mean was used to normalise the over- dispersed distribution of worm intensity. Infections were classified as light, moderate and heavy according to WHO criteria given in Table 2(15). For hookworm, 2,000 and 5,000 epg were used to define the lower boundaries of moderate and heavy infections, respectively. Differences between prevalence were tested by Chi square statistic and means were compared using Student's t statistic. Associations between parasitic infections were investigated using 2x2 contingency tables for each combination of parasites. The association between parasitic infections was measured using the ratio of prevalence and tested using the Chi square statistic

RESULTS

The demographic characteristics of the study population are shown in Table 1.

Table 1

Characteristics of study sample

Age (years)	Percentage
<7	1.3
7.0 - 8.9	20.4
9.0-10.9	44.1
11.0-12.9	26.0
>13	8.2
Sex:	
Males	51.8
Females	48.2
Residence:	
Rural	80.6
Urban	19.4

Late school enrolment and progressive drop-out of girls characterise this school population. The children were heavily infected with parasites (Table 2). The prevalence of intestinal helminth infections was 72%, 94% and 96% for A. *lumbricoides*, *T. trichiura* and hookworm, respectively. Thirty per cent of the children tested positive for haematuria. Malaria parasites were found in 61% of the children though parasitaemia was of low density with 47 % of children having less than 1,000 parasites per microliter of blood and only one per cent with more than 5,000 parasites per microliter. All infections were *P. falciparum*. *P. malariae* was identified in less than 5% of slides.

Table 2

Prevalence and intensity categories of intestinal helminths, malaria and haematuria

	A. lumbricoides	*T. trichiura*	Hookworms	Malaria		Haematuria	
Prevalence	72.0%	96.0%	93.7%		60.6%		30.5%
Intensity (epg)				(Parasite/µl of blood)			
Negative	28.0%	4.0%	6.3%	0	39.4%	Negative	69.5%
Light	51.7%	54.9%	74.7%	1-999	47.0%	Microhaematuria+	16.0%
Moderate	20.2%	40.1%	15.5%	1000-4999	12.6%	Microhaematuria++	11.0%
Heavy	0.1%	1.0%	3.5%	5000+	1.0%	Visual haematuria	3.5%

A. Lumbricoides	*T. trichiura*	Hookworms
Light=1-4999 epg	Light=1-999 epg	Light=1-1999 epg
Mod=5000-49999 epg	Mod=1000-9999 epg	Mod=2000-4999 epg
Heavy=50000+ epg	Heavy=10000+ epg	Heavy=5000+ epg

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Table 3

Prevalence of parasitic infections by sex, age and residence

	Boys n=1851	Girls n=1720	Age<10 n=1565	Age >=10 n=2905	Rural n=1872	Urban n=700	All n=3578
A. lumbricoides							
any infection	73.4	70.4	74.4	70.0**			72.0
moderate+heavy infection*	21.9	18.6	23.1	18.0***			20.3
T. trichiura							
any infection	95.9	96.1	96.4	95.7			96.0
moderate+heavy infection*	42.5	39.6	43.2	39.3†			41.1
Hookworm							
any infection	94.7	92.7*	93.4	94.1	94.2	91.8*	93.7
moderate+heavy infection*	21.1	16.8**	18.4	19.4	21.8	7.4***	19.0
Malaria							
any parasitaemia	60.6	60.7	45.7	54.3	62.4	91.8*	60.6
>1,000 parasites/µl	13.8	13.4	15.8	11.7***	13.8	7.4*	13.6
Total haematuria	32.6	28.1**	29.0	31.6			30.5
Visual haematuria	4.8	2.2***	3.5	3.5	3.8	2.2*	3.5
Microhaematuria#	28.4	26.3	26.8	28.5	31.3	16.0***	27.0

***p<0.001 **p<0.01 *p<0.05

* defined as in Table 2 #+ and ++ categories combined

Hookworm infection was more prevalent in boys (Table 3). Visual haematuria was 4.8% in boys and 2.2% in girls giving a relative prevalence of 2.21 for boys (95% CI=1, 51-3, 22). Though less dramatic microhaematuria was also more prevalent in boys.

Prevalence and intensity of *A. lumbricoides* infection and intensity of *T. trichiura* was slightly lower in older children (Table 3). Malaria parasitaemia (>1,000 parasites/microliter) was also lighter in younger children (p<0.001). Prevalence and intensity of hookworm and *S. haematobium* infections did not differ by age.

The prevalence of malaria parasitaemia, hookworm infection and haematuria was smaller in urban than rural schoolchildren (Table 3). These differences were smaller for malaria than for hookworm infection and haematuria.

Multiple infections with helminths were common. Only one child was negative for all the parasites. Infection with all three helminths was observed in 67.2% of children compared to 28.0% with double infection. The association between each pair of geohelminths, both for prevalence and intensity, was higher than expected by chance (Table 4).

Children free from infection due to any one of the geohelminths were more likely to be free from infection of any of the other two helminths. The pairwise relative prevalence of not being infected varies between 2.0 (CI= 1.44 - 2.78) for *A. lumbricoides* and *T. trichiura* and 2.97 (CI= 1.95-4.31) for *T. trichiura* and the hookworms. In contrast there was a negative association between hookworm infection and haematuria (Table 5).

Table 4

Association between geohelminth infections categories

	n	*Trichuris* <1,000 epg	≥1,000 epg
Hookworm			
<2,000 epg	2783	65.9%	34.1%
≥2,000 epg	653	28.9%	71.1%

	n	*Ascaris* <5,000 epg	≥5,000 epg
Trichuris			
<1,000 epg	2024	91.0%	9.0%
≥1,000 epg	1412	63.5%	36.5%

	n	Hookworm <2,000 epg	≥2,000 epg
Ascaris			
<5,000	2739	85.8%	14.2%
≥5,000 epg	697	62.0%	38.0%

p<0.0001

Table 5

Hookworm infection by haematuria

	n	Haematuria negative	Positive
Hookworm			
Negative	215	56.8%	43.2%
Positive	3214	70.2%	29.8%

p<0.001

DISCUSSION

We have documented a very heavy burden of parasitic infection in Zanzibari school children. A number of other

recent studies in East Africa have found high rates of geohelminth infections, especially in coastal areas(16-18). Although the prevalence of infection with any helminth varies among these studies, they have all found, as we did, that most schoolchildren are infected with at least one helminth. However, the intensity of geohelminth infections from our survey, particularly of hookworms, is striking. The rates are higher than in other studies of African school children and from other parts of the world such as Jamaica(5), India(19) and China(20).

We also found that 31% of school children in our study had haematuria, a reliable indicator of schistosomiasis infection in this population(12). This is the average prevalence of haematuria maintained by the ongoing schistosomiasis control programme, based on yearly selective treatment of children in schools where haematuria is more than 20%. This survey was performed about ten months after the previous treatment with praziquantel. The 54% prevalence estimated in 1986, before the intervention, is similar to the prevalence found in other studies in sub-Saharan Africa, such as the 76.4% and 55% prevalence found respectively in Niger(21) and in Ethiopia(18). The chronic loss of blood in urine was recognised by Prual *et al*(21) to be an important cause of iron-deficiency anaemia.

We found no difference in either prevalence or intensity of *A. lumbricoides* and *T. trichiura* infection by sex though boys had slightly heavier hookworm infections. These results are in agreement with previous studies(16,22).

We further found that visual haematuria in boys was more than twice as prevalent in girls. This is in agreement with several other studies which have found higher rates of schistosomiasis among boys, possibly reflecting higher exposure to infection due to closer contact with water bodies(18,23,24).

We found that *A. lumbricoides* and *T. trichiura* infections decreased after the age ten but there was no change in prevalence of hookworm infection with age. Others have found that intensity of infection with *A. lumbricoides* and *T. trichiura* in school children remain constant with age whereas that of hookworm infection increases steadily with age(25).

Finally, we looked at the interaction between different parasites. We found a strong positive correlation among the three helminths as previously demonstrated by Booth *et al*(26) with data from the same environment. Although we expected *A. lumbricoides* and *T. trichiura* to be most strongly associated, our findings suggest that frequent contacts with soil contaminated with human faeces, equally expose the children to all the three geohelminth infections regardless of oral or percutaneous transmission. Although *Necator americanus* is the predominant hookworm species in Pemba, 27% of children are infected with *Ancylostoma duodenale*(27), and its possible faecal-oral transmission may account in part for the association with the other geohelminths. The negative correlation between hookworm infection and schistosomiasis is surprising. Children infected with hookworms are less likely to have haematuria. The expected positive correlation may have been reversed in Pemba as a consequence of the periodic treatment with praziquantel that children have been receiving since 1986.

We also looked at the difference between children attending schools in urban and rural areas. Generally, we found the prevalence to be the same but with an increase in intensity of the geohelminths in rural areas. These findings contrast with previous results(28) in the same population, in a smaller sample size. It is interesting to note that in rural areas where transmission is more intense and prevalence very high, higher exposure is reflected only by higher intensity of infection. As expected we also found a large difference in schistosomiasis, with the prevalence of haematuria almost doubled in rural children. The strong association between schistosomiasis and water bodies and the availability of piped water in urban areas are likely to account for this difference. Malaria parasitaemia was also higher in rural school children. Malaria inoculation rate might be lower in urban areas because of lower mosquito density and the more common use of bednets(29). Urban children have also better access to medication than children living in rural areas.

In conclusion, the parasite burden is distributed broadly and although there is a difference between urban and rural areas, parasitic infections are still high in both. The burden of disease associated with parasitic infections has been shown to have a negative impact on iron status of school children in this population(11).The iron status of our population was very poor as illustrated by 62% of children having anaemia and 78% of the anaemia being iron deficiency type. Iron-deficiency anaemia is an important public health problem in Zanzibar schoolchildren(11). Although schistosomiasis and malaria can contribute to its aetiology, the most important cause in school children are intestinal blood loss due to hookworm infection and poor diet(11,30).

Our findings have a number of implications for public health intervention in Zanzibar. First, the high prevalence of intestinal helminthiasis and schistosomiasis among school children justifies the implementation of school-based interventions such as periodic anthelminthic treatment. Because of the enormously high prevalence and the availability of safe and inexpensive single-dose drugs, screening individuals for selective treatment might not be cost-effective. Indicators for morbidity such as intensity of infection and visual haematuria on a sample of the population should be sufficient for monitoring and guiding control programmes. Similarly, the relatively even distribution by age and by sex does not justify a priori selection of school children for treatment. However, the large decrease of school attendance in girls with increasing age might cause a large proportion of older girls to be missed out through school-based interventions in Zanzibar. This is an important and unresolved challenge because uncontrolled parasitic infection will cause women to attain their reproductive lifes while at a high risk of anaemia.

These results advocate for an integrated control programme which takes into account other endemic

diseases like filariasis, that can be controlled using similar strategies. The results of this study have led to the preparation of a Plan of Action for the integrated control of intestinal helminths, filariasis, malaria and schistosomiasis by the Ministry of Health of Zanzibar with the technical support from the Division for Control of Tropical Diseases of the World Health Organization(31). Malaria control is an especially complex issue, but it can be implemented in an Integrated Disease Control Programme where resources are shared and intervention is carried out at both central and community levels with the supervision and co-ordination from the District Health Management Team(31).

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Estimating Reactivity in Direct Observation Studies of Health Behaviors

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Direct observation techniques are increasingly being used to measure human behavior as part of health research and intervention studies. One problem with this approach that has not been well-explored is the effect of the observer's presence on the behaviors being observed, the reactivity effect. This article examines reactivity effects in a direct observation study of child care and feeding practices in rural Nepal. Trained field workers conducted day-long structured observations in 160 households, seven times per household. Our results indicate that the first day of observation is more reactive than later days. A number of individual behaviors changed in frequency from observation day to observation day, including punishment of the child and method of serving food to the child. Observation days with higher levels of reactivity showed an increase in the number of positive health behaviors and a decrease in the number of socially negative behaviors. We conclude that the validity of direct observation studies of behavior can be enhanced by examining and controlling for reactivity effects.

Key words: direct observation, reactivity, measurement, health behaviors, Nepal

Direct observation is a method for assessing human behavior in natural settings. In recent years, observational methods have begun to be incorporated into health interventions and health-related studies as a means of identifying behaviors which place individuals or households at increased risk for morbidity and mortality, and for evaluating the effectiveness of programs to promote positive health behaviors. For instance, structured observations have been used to estimate intra-household food allocation patterns in Nepal (Gittelsohn 1991), dietary management of diarrhea in Peru (Bentley 1991), and sanitary behaviors in Bangladesh (Stanton and Clemens 1987) and in Burkina Faso (Curtis 1993).

The primary advantage of direct observation over other methods for acquiring information on how humans behave is that direct observation captures actual behavior. Most methods for estimating human behavior rely on reports of previous behavior by the human actor or a proxy (as in the mother reporting what her child ate). For many reasons, there is frequently a big difference between what people say they do (or did) and what they actually do (Ricci *et al.* 1995). People may forget what they did, or prefer to answer with what is considered locally appropriate, or what they think the interviewer would like to hear (Bentley *et al.* 1994). A second advantage of structured observations lies in their ability to capture the context of human behavior. Much human behavior occurs as a result of or precedes other behaviors. This kind of information, sometimes termed the "behavioral stream," can be captured best using direct observations.

Despite these advantages, a number of concerns have been raised about the validity of direct observation data. These concerns were laid out by Haynes (1978) and include: 1) excessive variance in observers (i.e., inaccuracy) or observation situations (e.g. sampling error); 2) inadequate or erroneous sampling procedures; 3) observer bias and drift; 4) inaccurate recording or coding methods; 5) inadequate development of codes; and 6) reactivity. This article will focus on the last concern.

What is reactivity? Reactivity occurs when actors change their behavior due to the presence of an observer. Baum *et al.* (1979) noted that "the presence of an observer constitutes a significant social stimulus which may cause subjects to alter their behaviors and which may elicit socially desirable responses. The investigator is then presented with an atypical sample of behavior which deviates from that manifested by the subject outside of the research setting." Of concern, then, is that these changes in behavior will create systematic bias in the data, particularly those behaviors of interest for the study. For instance, in a study of food allocation, family members

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might change their dietary intake (e.g., eating more expensive foods) in response to the presence of the observer.

Reactivity can potentially effect the validity of a study. In intervention studies, it can compromise internal validity by directly affecting measurement of the dependent variable since reactive behavior changes may be wrongly attributed to an experimental intervention (Kent and Foster 1977). It can effect the generalizability (external validity) of studies by leading to erroneous study findings.

The importance of investigating the phenomenon of reactivity has not escaped other investigators. Probably the single most comprehensive body of work comes from the field of psychology and behavioral sciences. Direct observation of human behavior is a hallmark of many psychological studies, including studies of children at play, the interactions between adults and children, of couples, and so on.

Psychologists have studied the phenomenon of reactivity for many years. Studies have varied considerably in terms of the paradigm used and according to the setting of the observations. Baum *et al.* (1979) in a review of 17 studies of adult-child interactions which had a reactivity component, found reactivity reported in three fourths of the studies. Most of the 17 studies were conducted in a laboratory (8/17) under controlled circumstances or in classrooms (5/17) — very few of the studies were conducted in the home environment (4/17). Baum *et al.* conclude from their review that reactivity was an issue — with certain behavioral biases more common than others. Adults reacted to the presence of observers with more positive and neutral verbal interactions and physical performance. There was no change in terms of negative categories of behavior. Children show no clear trend in reactivity, and the authors conclude that there are not enough studies of reactivity in children yet to justify conclusions. In the 17 studies, three different paradigms for measuring reactivity were used:

> 1) Habituation: where behavioral changes over time indicate adjustment to the presence of the observer. It is assumed that such changes reflect initial reactivity and subsequent adaptation to the observer's presence.
>
> 2) Conspicuousness: which involves a comparison of procedures which vary the obviousness of the observer's presence to the person(s) being observed (i.e. observer present in room, use of a video-tape recorder, observer behind one-way mirror).
>
> 3) Awareness: which involves manipulation of subject's consciousness of being observed (i.e. telling the subject they will be observed and when, telling the subject they will be observed but not at what times, not telling the subject they will be observed).

The awareness paradigm was the most common means of testing reactivity effects in these studies. All three of these paradigms have disadvantages. Habituation relies heavily on assumptions of patterns of reactivity, and how these patterns will change over time. The conspicuousness paradigm is useful primarily in highly controlled settings, such as the laboratory. In a study of couples, Carpenter and Merkel (1988) found no difference in reactivity related to the use of a one-way mirror, compared to audio-taping or video-taping. Manipulating awareness of being observed has a number of obvious ethical concerns.

The habituation paradigm deserves further discussion. There is evidence to support the premise that initial observations are reactive, but later observations are not (or much less so). Several investigators (Haynes and Follingstad 1977; Moos 1968; Purcell and Brady 1966) have found that reactivity decreases as observation sessions continue. Haynes (1978) observes, "Reactivity may also be inferred from a slope in the resultant data, although slope may also be a function of other determinants. A gradual increase or decrease in rate may suggest that initial levels of the behavior were a function of the reactivity of the observation process and that, because of habituation, later behavior rates are a more accurate representation of the "true" rate." In one study, while reactive positive behaviors decreased as subjects became more accustomed to being observed, they immediately increased when a new observer was introduced (Zegiob *et al.* 1979).

Haynes (1978) concludes, "*under some conditions* the observation process results in a significant change in the behavior of target subjects. The task of the behavior analyst is to understand factors determining reactivity and methods available to reduce, control or predict its effects." Despite the work that has been conducted, there are large gaps in our knowledge of reactivity effects in observational studies of human behavior. A gap that directly applies to the topic of this article lies in the absence of information on reactivity effects in regard to health behaviors. To our knowledge, no studies have been conducted in this area. In addition, few studies of reactivity have been conducted in naturalistic settings. This is a critical omission when doing health-behavior studies. Health behaviors are expressed within the household in the context of daily life, and would be difficult to examine in highly controlled settings, especially in developing country settings.

The overall purpose of this paper, then, is to examine reactivity effects in the context of a household-level health behavior study in a community setting. The reactivity data here use the habituation paradigm, where trends in the frequency of behaviors are viewed as evidence of reactivity. We seek to answer the following questions:

> 1) What is the pattern of reactivity in direct observations of health behaviors? Are first observations more reactive than later observations?
>
> 2) Over multiple days of observation, will there be stabilizing trends in the frequency of key health-related behaviors (indicating habituation to the presence of the observer)? When does this occur?
>
> 3) Do more reactive days of observation show different patterns of health behaviors than less reactive days of observation?
>
> 4) Do more reactive days of observation show different patterns of socially positive and negative behaviors than less reactive days of observation?
>
> 5) What is the significance of our findings for other investigators planning to use observational techniques to explore and measure health behaviors?

Methods

The reactivity data presented in this article were collected as part of a study looking at the behavioral determinants of vitamin A deficiency among children in rural Nepalese households. The study was conducted in Sarlahi district in the

rural Terai (lowland) region of the country. The population of the area is predominantly Hindu, living primarily at a subsistence agricultural level.

Seven day-long observations were conducted in each of 162 study households during the period from April, 1993, to May, 1994. Observations in a household were spaced roughly two months apart. 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). 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 2-5 years old and that of caregivers for that child. However, observations were recorded for other household members, particularly other children. 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 am) until the focal child went to sleep for the night and the evening meal had been consumed (usually around 8 pm). The observer took a 3-4 hour break in the middle of the day.

Observed behaviors were recorded as 5 letter mnemonic codes, each representing a Nepali phrase (e.g. AFKLI stood for *Aaphno khaana linchha*, or "person takes their own food"). In total, 110 different behavioral codes were developed for the study. The codes covered seven topical areas: child care, food preparation and serving, general, observer-related, personal hygiene and health, conversation, and work.

In total, 1101 days of observation were collected. Several households were dropped from the study due to migration, and three households refused to continue with the observations after the first visit. An average of 363 lines of observation were collected from each study household for each day of observation, yielding a total of 399,730 total lines of observation.

Limiting Reactivity

Observers were trained to behave in a manner which would reduce overall reactivity as much as possible. This included visiting the household for a short period prior to beginning actual observations, training on how to be inconspicuous, not to engage in child care activities, and in particular, not to participate in household food behaviors.

Operationalizing Reactivity

Measurement of reactivity was built into the study from the outset. Two systems for estimating level of reactivity were used: 1) A set of codes for reactive behaviors were created; and 2) An overall estimate of level of reactivity was made by the observer at the end of the observation.

Three reactivity codes were created: Low, Moderate and High. Low-level reactivity behaviors, most commonly involved watching the observer. The Moderate-level reactivity code was used when the observer's work was interrupted by others, often in the form of conversation. This included instances of being asked for favors, medicine, etc. The High-level reactivity code was used to indicate a behavior with an obvious high level of reactivity to the presence of the observer. This code was used when actors changed behaviors of interest to the study — especially feeding, preparation of special foods and child care. The decision on whether or not to use this code was made initially by the observer, but later corroborated by person(s) observed.

A summary estimate of reactivity was made by the observer at the end of the observation. Observers were asked to score reactivity of the observation along a scale of 1 to 5, with 1 indicating little to no reactivity and 5 indicating very high reactivity, where household members altered behaviors of interest to the study (i.e. feeding and child care) (Table 1).

Table 2 presents the mean number of times each reactivity code was used for all the days of observation combined, as well as the mean summary estimate of reactivity made by the observer. The Low-level reactivity code (LRC) was used an average of 0.757 times per observation visit, while the Moderate-level reactivity code (MRC) was used at about half that frequency. The code indicating highly reactive behavior (HRC) was very rarely used. The mean of the Overall Observation Day Reactivity Estimate (OODREACT) was 1.605 (on a scale of 1 to 5), indicating a very low level of reactivity, when

Table 1. Codes for Summary Estimates of Reactivity by Household Members

Code	Definition
1	Very minimal contact with the observer. Household members may exchange greetings with the observer, but otherwise do not noticeably change behaviors.
2	Interacts minimally with the observer (e.g. 1-2 times asking questions). There may be a few minor changes in behavior (e.g. offering food to the observer, crowd gathers during observation).
3	Interacts 3-5 times with the observer during the day. Women may hide their face while doing activities (may stay in the house). Food usually offered to observer.
4	Interacts with the observer more than 5 times. Women may hide breast-feeding or other eating from the observer. Foods may be prepared especially for the observer.
5	Change in household feeding or child care behaviors. Foods may be prepared especially for the observer. This is different from #4 because the household members verbally verified the change in behavior is due to the presence of the observer. Foods may appear to be not allocated to household members so they can be given to the observer.

Table 2. Frequency of Three Behavior-level Reactivity Code Usage and the Reactivity Summary Estimate (N=1101 Day-long Observations)

Reactivity Code	Mean	STD	Minimum	Maximum
Low-level reactive behavior	0.757	1.663	0	17
Moderate-level reactive behavior	0.321	0.774	0	6
High-level reactive behavior	0.090	0.433	0	6
Overall observation day reactivity est.	1.612	0.828	1	5

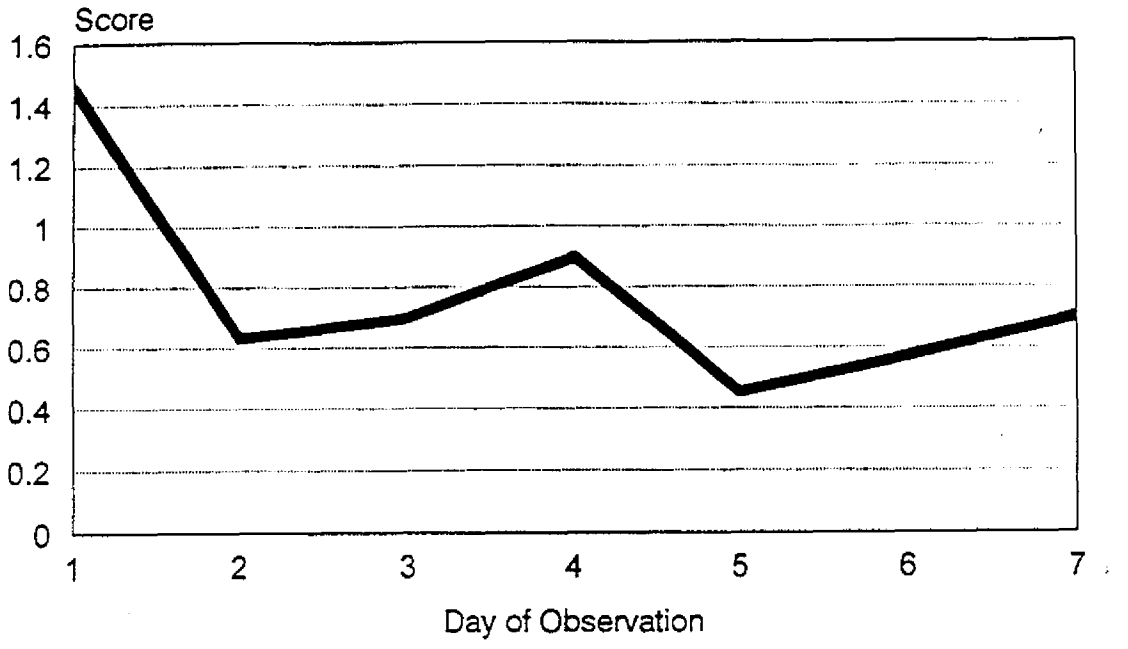

Figure 1. Change in Reactivity Score by Day of Observation

compared against an average of 363 recorded behaviors per observation-day.

A principal components factor analysis was conducted on the mean daily scores for Low, Moderate and High-level reactivity and on the Overall Observation Day Reactivity Estimate to determine whether these items could be combined into a scale (Mahoney *et al.* 1995). Two factors had eigenvalues greater than 1: (a) a combination of the Low-level reactivity code, Moderate-level reactivity code and the Household Reactivity Summary Estimate, and (b) the High-level reactivity code alone. In total, these two factors account for 67.8% of the variance. We decided to combine all four variables into a single reactivity score, using the standardized scoring coefficients produced for the first factor as weightings. The final reactivity score was calculated as follows:

Final Reactivity Score = (# LRC * 0.45791) + (# OODREACT * 0.43537) + (# MRC * 0.41951) + (# HRC * 0.29307) - 0.43537

The lowest possible value of the final reactivity score (0.43537) was subtracted in order to create a scale with the base score of 0. The data were then divided into terciles for analysis. In this manner, each observation day was described in terms of its overall level of reactivity.

It was necessary to eliminate several behaviors before further analysis could be conducted. As the observations were conducted over a year, there is a strong possibility that frequencies of certain behaviors would be effected by changes in season. Twenty-two such codes were dropped from the analysis. Codes dropped due to possible seasonal effects included gathering wild greens, work in agricultural fields, gathering firewood, and so on. Another set of codes dropped from the analysis were those with frequencies that might be affected by maturation of the child over the course of the study. Focus child ages at the initiation of the study ranged from 2 to 5 years. Certain of these behaviors, such as the child defecating or urinating in plain sight of the observer, are less likely to occur as the child grows older.

Results

Patterns of Reactivity

Household observations were characterized by relatively high initial reactivity, followed by much less reactivity from the second observation day on. Figure 1 presents the mean Final Reactivity Score for each of the seven days of observation. The Pearson correlation coefficient for this relationship is -0.175 ($p<0.0001$). Therefore, using our observer-generated codes for reactivity, it appears that the initial day of observation is more reactive than later days.

Changes in the Frequency of Key Behaviors

Reactivity can also be examined indirectly by examining changes in the frequency of behaviors from observation visit to observation visit; the key assumption of the habituation paradigm. Given the finding of higher initial reactivity based on specific reactive behaviors recorded by observers, it is important to see how this may or may not relate to other key behaviors.

Figures 2-5 indicate trends in the daily frequency of selected behaviors involving the focus child. Physical punishment of the child did vary significantly by day of observation, with the amount of mild ($r=-.132$, $p<0.0001$) and moderate beating ($r=-.124$, $p<0.0001$) of the child decreasing with later observations

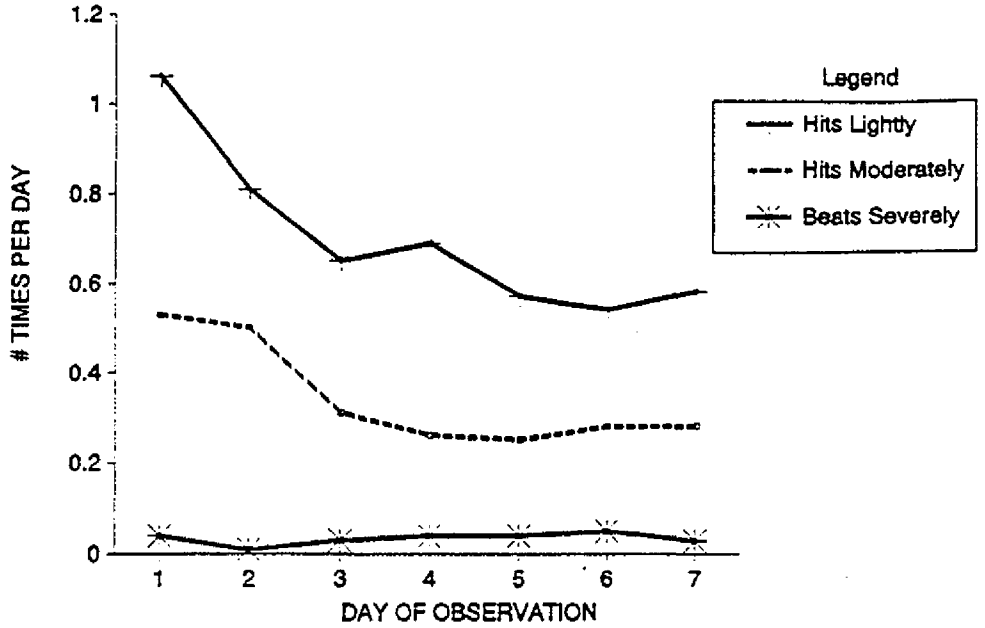

Figure 2. Trends in physical punishment involving the focus child

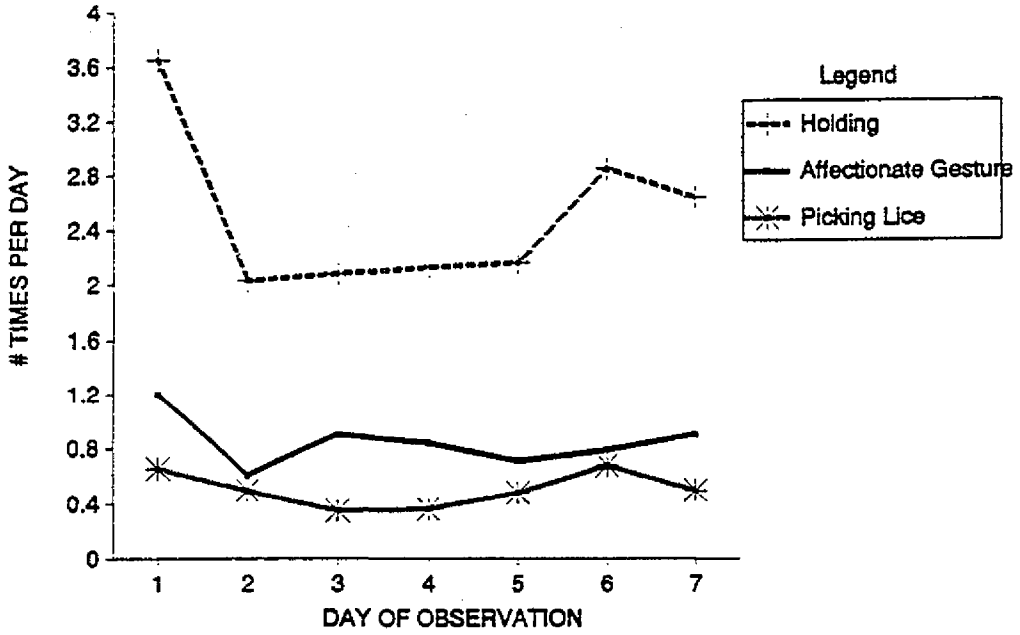

Figure 3. Trends in displays of affection involving the focus child

(Figure 2). No significant trend was observed in the frequency of harsh beating, a fairly rare occurrence. Nor was any significant trend found in the frequency of individual behaviors relating to displays of affection (Figure 3), including affectionate gestures to the child, holding the child, or picking lice from the child's hair.

In terms of food serving, changes were observed in the manner in which food was served to the child from one observation to the next. In all instances where the focus child received food (Figure 4), there is a tendency for the child to reduce the frequency of self-serving ($r=-.104$, $p<0.0006$), to be served automatically less often ($r=-.246$, $p<0.0001$) and to be asked if they want food more often ($r=.083$, $p<0.0056$) in later observations...No significant trend is observed in the frequency of times the focus child asked for food and was served food. Nor were any significant trends observed from visit to visit in

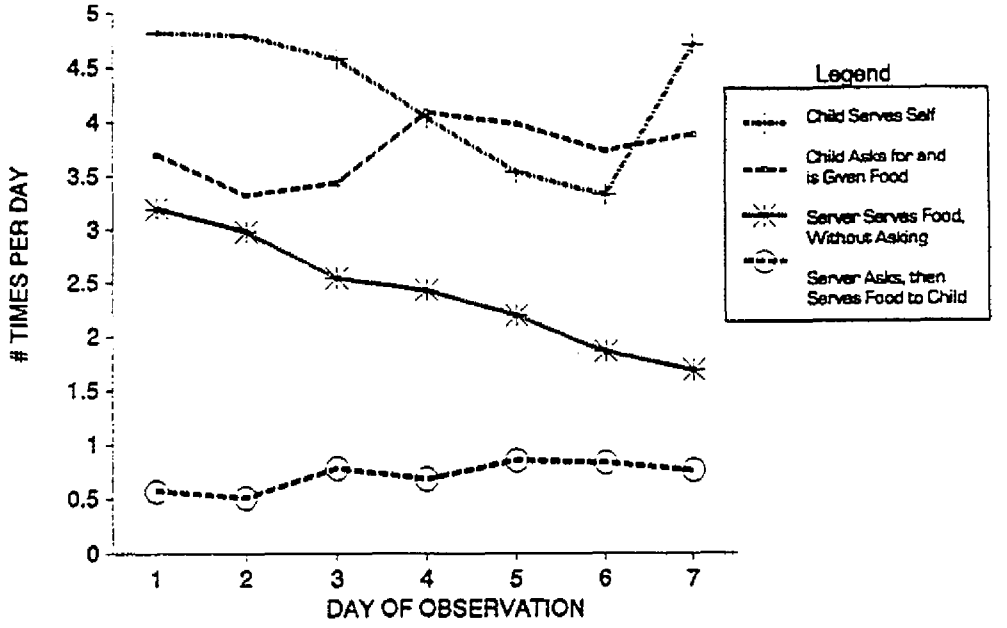

Figure 4. Trends in how food is served to the focus child

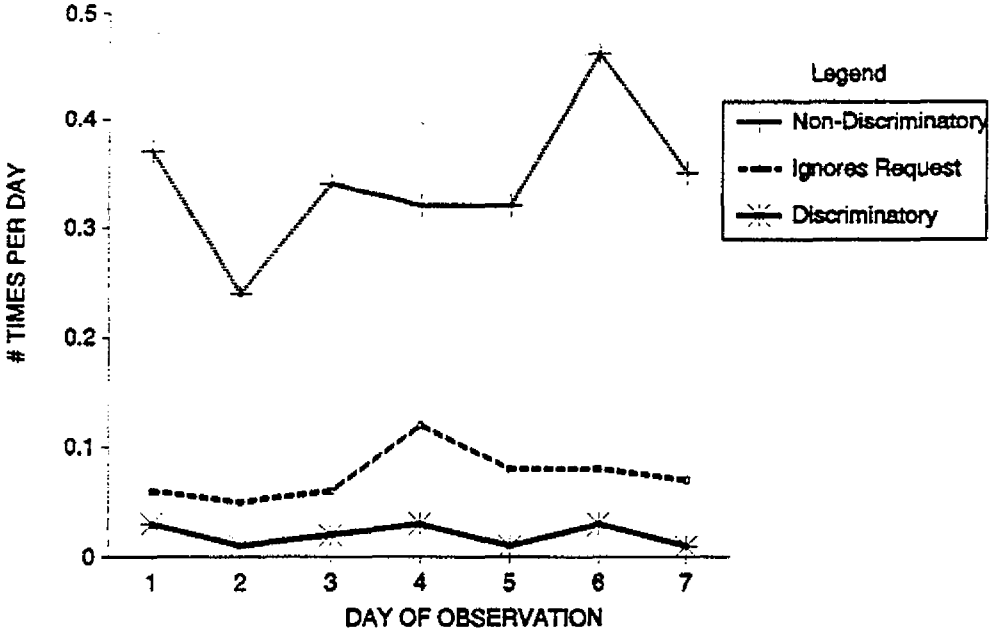

Figure 5. Trends in types of food serving refusals to the focus child

terms of the frequency of food serving refusals, where the focus child asked for food but was refused (Figure 5).

Reactivity Effects on Health Behaviors

How does reactivity effect the health behaviors of household members being observed? This question is of central importance to investigators and program planners who wish to use observation to obtain estimates of health-related practices within the household. In order to examine reactivity effects on health behaviors in this study, we developed two additive scales: positive health behaviors and negative health behaviors. From the list of household behaviors, 15 positive and 7 negative health-related behaviors were identified. What was considered positive or negative was determined from a biomedical perspective. Positive health behaviors included: encouraging

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Table 3. Scaled Positive and Negative Health Behavior Scores by Day of Observation Reactivity Effect

	All Household Behaviors							Behaviors Involving the Focus Child						
	Reactivity (TOTREACTt)						ANOVA	Reactivity (TOTREACT)						ANOVA
	LOW		MED		HIGH			LOW		MED		HIGH		
	Mean	Std Error	Mean	Std Error	Mean	Std Error	F	Mean	Std Error	Mean	Std Error	Mean	Std Error	F
Positive Health Behaviors														
	1.000	0.022	1.002	0.028	0.970	0.024	0.54	1.000	0.053	1.059	0.058	1.187	0.056	3.11*
Negative Health Behaviors														
	1.000	0.042	0.979	0.046	0.937	0.046	0.55	1.004	0.101	1.172	0.113	1.053	0.103	0.63

* $p<0.05$. Reported as ratios to low reactivity tercile summed over all days of observation

Table 4. Scaled Positive and Negative Social Behavior Scores by Day of Observation Reactivity Effect

	All Household Behaviors							Behaviors Involving the Focus Child						
	Reactivity (TOTREACT)						ANOVA	Reactivity (TOTREACT)						ANOVA
	LOW		MED		HIGH			LOW		MED		HIGH		
	Mean	Std Error	Mean	Std Error	Mean	Std Error	F	Mean	Std Error	Mean	Std Error	Mean	Std Error	F
Positive Social Behaviors														
	1.000	0.022	0.969	0.024	1.006	0.024	0.68	1.000	0.033	0.993	0.033	1.003	0.032	0.03
Negative Social Behaviors														
	1.000	0.048	1.140	0.052	0.988	0.045	2.83	1.000	0.052	1.018	0.051	0.840	0.042	4.07*

* $p<0.05$. Reported as ratios to low reactivity tercile summed over all days of observation

someone to eat more food, picking lice, brushing teeth, cleaning up feces in a sanitary manner, and using soap for cleaning/washing. Negative health behaviors included beating someone moderately or strongly, refusing requests for food (in a discriminatory fashion), injuries, and cleaning up feces in an unsanitary fashion.

Total observation-day scores for both scales are reported as a ratio to the score for the least reactive days of observation. Final Reactivity Scores have been divided into terciles using the TOTREACT variable. A number greater than one indicates a relatively higher frequency of health-related behaviors (compared to low reactivity days), while a number less than one indicates the opposite. From Table 3, it appears that there is a trend towards decreasing frequency of both positive and negative health behaviors with more reactive observation days at the household level, however these differences were not significant using analysis of variance procedures. However, when health scores were calculated using behavioral events only involving the focus child, the analysis of variance procedure did show a significant increase in positive health behaviors with greater reactivity.

Reactivity Effects on Positive/Negative Social Behaviors

As reported earlier, the literature indicates that socially positive behaviors are more likely to occur in initial, more reactive observation periods. Behaviors considered socially negative are presumed to be less frequent in those initial periods. Twenty-three socially positive and nine socially negative behaviors were identified based on our working knowledge of the culture and setting. Socially positive behaviors included: affectionate gestures, holding the child, picking lice, encouraging someone to eat, cleaning up feces, and teaching someone. Socially negative behaviors included beating someone, refusing to give someone food (discriminatory), stealing food from another, fighting, and quarreling.

Again, scores for both scales are reported as a ratio to the score for the least reactive days of observation. A number greater than one indicates a relatively higher frequency of social behaviors (compared to low reactivity days), while a number less than one indicates the opposite. From Table 4, it appears that there is no specific trend towards increasing or decreasing positive or negative social behaviors with more reactive observation days at the household level. On the other hand, there is a significant trend towards decreasing the socially negative behaviors with more reactive days when just the focus child is considered.

Discussion and Conclusion

We were able to operationalize a reactivity variable that proved useful in evaluating the quality of our observational dataset. Overall, our measures indicated that the level of reactivity was low. Of the seven days of observation, the initial day was considerably more reactive than other successive days.

After eliminating a number of behaviors that are effected by season and growth or aging of the child, trends in the frequency of remaining specific key behaviors were observed over the course of the observation period. In terms of physical punishment of children, it is important to observe that our findings are in the opposite direction that one would expect from the literature. Children were punished much more frequently in initial observations than in later observations. This trend may or may not reflect reactivity to the presence of an observer. It is possible that the focus child was punished frequently in the beginning of the observations due to the perception that he or she was disturbing the observer. On the other hand, this trend may reflect an aging effect, where younger children make more frequent mistakes and need to be disciplined more often. Changes in patterns of food serving to the focus child were observed and may also be related to an unanticipated

maturation effect. As children grow older, they may be less likely serve themselves or to be automatically served — and more likely to be asked if they want food.

While no appreciable reactivity effects were observed in terms of overall scores of positive and negative social behaviors or negative and positive health behaviors when looking at the entire household, significant differences were observed when just behaviors involving the focus child were considered. The increase in the number of positive health behaviors related to more reactive days is believable as many of the behaviors involved cleaning, an activity that household members are likely to perform in the presence of an outsider. The significant decrease in the score for negative social behaviors in more reactive observation-days agrees with findings in the psychological literature on reactivity. It is interesting that the negative social behavior scale moves in the opposite direction of some of its component variables, namely physical punishment of the child. On closer inspection of the data, quarreling, scolding and fights involving the focus child occur much more frequently than do episodes of physical punishment and account for this reversal. It appears that as levels of punishment go down, negative social behavior on the part of the focus child increases. We interpret these results to mean as we moved on with our observations in a household, reactivity decreased and behaviors became more normal.

This study in Nepal has several implications for studies using direct observations of health behavior. First, reactivity effects may very well play a role in patterns of health behaviors observed, especially positive health behaviors. Second, reactivity appears to effect the frequency of negative social behaviors. These, in turn, may be related to patterns of health behaviors in the household. Third, these effects appear stronger among selected household members (in our case, among young children). It is also possible that since we were focusing our observations on young children, we only had sufficient numbers of behaviors recorded to detect differences for that age group. Finally, most of the reactivity appears to occur during the first day of observation.

This last finding is of key importance to investigators and program planners intending to conduct direct-observation studies of health behavior. The social science public health literature provides numerous examples of the "single shot" observation study, where households or individuals are monitored on one occasion only. This approach to doing direct observation studies should be reconsidered. When multiple episodes are collected for the same individual(s), elimination of the first observation day's behavioral data, or standardizing it according to successive days is an option to control for reactivity effects.

It should be understood that our most reactive observation days were actually only **relatively** more reactive than others — there is no way to objectively state one observation visit was reactive while another was not. We conclude that direct observation provides a replicable and robust means for estimating behaviors of importance for health behavior studies. The validity of the method can be enhanced by measuring and controlling for reactivity effects.

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CULTURAL FACTORS, CALORIC INTAKE AND MICRONUTRIENT SUFFICIENCY IN RURAL NEPALI HOUSEHOLDS

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Abstract—This study examined the allocation of food within 105 Nepali households using a combination of recall and observation methods. While a relationship exists between caloric intake and sufficiency of intake of several key micronutrients (i.e., beta carotene, vitamin C and iron) for the study population as a whole, this relationship is weaker for certain subgroups. In particular, micronutrient intakes of adolescent girls and adult women are much less likely to be tied to total caloric consumption when compared with the intakes of other household members. This gender differential appears linked in part to specific food beliefs and practices that tend to reduce women's consumption of micronutrient-rich foods, such as dietary restrictions during menstruation, pregnancy and lactation. Overlapping with these beliefs and practices, an overall pattern of disfavoritism of females in the intrahousehold allocation of food is evident in the study communities. While staple food items (i.e. rice, lentil soup, bread, etc.) are distributed fairly equally, side dishes usually containing a higher proportion of micronutrients (i.e. vegetables, meat, yogurt, *ghee*, etc.) are often preferentially allocated to valued household members, including adult males and small children (of both sexes). © 1997 Elsevier Science Ltd

Key words—micronutrient sufficiency, caloric sufficiency, food beliefs and practices, intrahousehold food allocation, diet, Nepal, gender

INTRODUCTION

This paper explores the relationship between caloric sufficiency and micronutrient sufficiency in rural Nepali households. For many years, a prevailing belief in the field of international nutrition was that one need only provide adequate calories and adequate micronutrient intakes would naturally follow [1]. Today, it is more widely accepted that adequate energy intake does not ensure adequate levels of micronutrients. Still, few studies have looked at this relationship within specific household and cultural contexts. The data presented here, based on a study of intrahousehold food distribution patterns and nutrient intake in Nepal conducted in 1986–87 [2], provide a case example examining the relationship between caloric and micronutrient intakes, and describe sociocultural factors which influence this relationship.

The conceptual framework employed portrays individual dietary intakes (and nutritional status) as part of an integrated process of food selection, acquisition, distribution and consumption. These features are, in turn, affected by a complex interplay of environmental, economic, and sociocultural factors at national, community, household and individual levels (Fig. 1).

The focus of this paper is on dietary outcomes, specifically, gender and age-based differentials in nutrient intake, and the sociocultural and behavioral factors that determine these patterns. In examining dietary patterns and their effects on the caloric and micronutrient intakes of households, the following questions were addressed:

(1) What cultural beliefs appear to influence the allocation of foods to members of rural Nepalese households?
(2) What is the relationship between energy and micronutrient intakes in this setting? Are diets sufficient in energy also sufficient in micronutrients?
(3) How does the sex and age of the individual affect this relationship?

BACKGROUND

Differential intrahousehold allocation of food: effects of gender and status

In most social settings an individual's status within the household is largely determined by three main variables: age, gender and kin relationship [2–6]. In South Asia, gender-based differentials in status are reported to reflect the *current* economic contribution of adult males versus adult females and the perceived *future* economic potential of the male child versus the female child [7]. Female children, in

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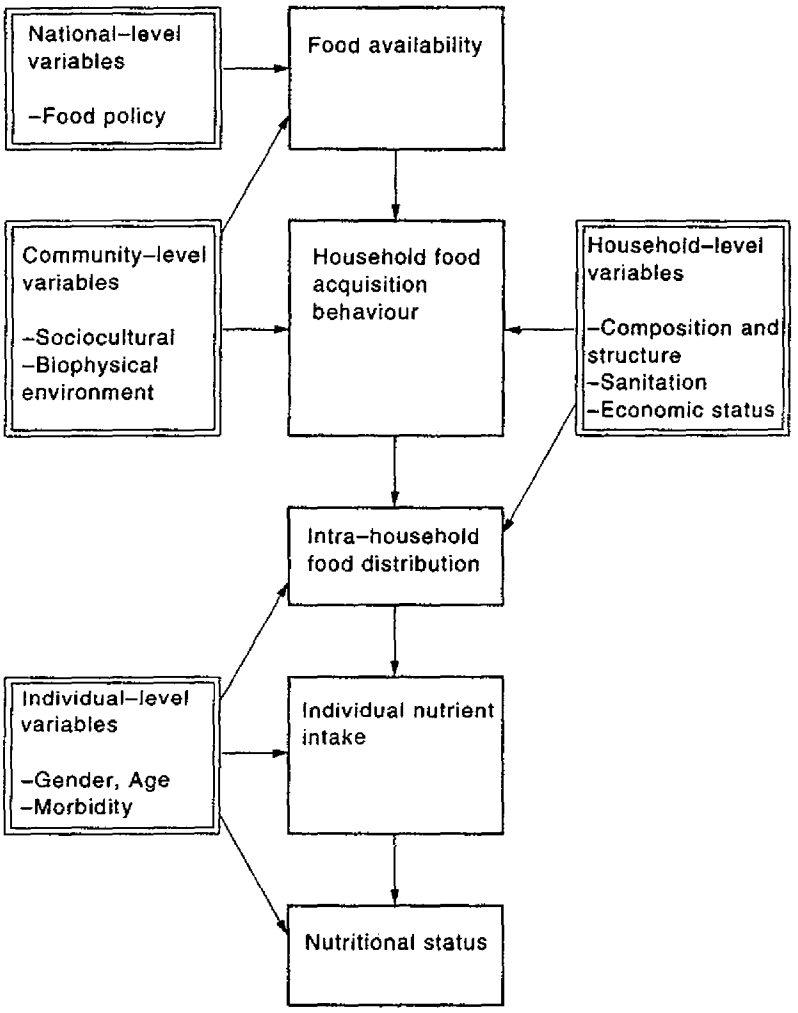

Fig. 1. "Food flow" model of intrahousehold food distribution.

these settings, are perceived to be an economic drain on the household. All inputs (e.g. food, education, health care) to female children are unlikely to be returned as they mature, except in labor extracted before they marry and leave the household. The lower value placed on women is social and cultural, as well as economic. Some investigators have noted that in Hindu societies women are viewed as polluting (particularly during menstruation) and as potentially threatening to the patrifocal system [8]. Bennett observes, "women are problematic—partly because of their association with sexuality and fertility and partly because they are perceived as destructive to agnatic solidarity" [7]. In a study of female discrimination in Punjab, Das Gupta [9] points out that discriminatory behavior does not always occur on a conscious level:

> Parents in a society may simply have internalized certain norms that lead them to give better care to their sons than their daughters, and excess female mortality may be an unintended consequence.

Within the household, the allocation of food, like other resources, varies according to culturally prescribed patterns. This is not a new concern. Pelto [10] observes, "It has long been recognized that malnourished children are often found in households where their siblings approach normal height and weight". Van Esterik [11] notes,

> There is sufficient evidence to say that food is not equally divided within households...the distribution of food reflects the order of precedence and social value of the food consumers.

Pinstrup-Anderson's [12] metaphor of intrahousehold food allocation as a "black box" is well known. In recent years, several researchers have attempted to describe the inside of this "black box", including the form of behaviors by which some household members are shown preference and others disfavored, as well as the economic, environmental and sociocultural determinants of food allocation patterns [1, 13–17].

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Gender differences in household food allocation have been observed in such diverse locales as Bangladesh [18], Brazil [19], Great Britain [20], the Ivory Coast [21], and the Sudan [22]. In South Asia, studies have identified several determinants of preferential food allocation. Preference in food allocation for adult males has been widely reported in India, Bangladesh and Nepal [2, 23, 24–26]. Much of the available evidence is based on either nutritional status data and/or health outcome data which show higher rates of morbidity and mortality in females [27–30], or by inference from stated beliefs and reported behavior [31–35]. In Punjab, Das Gupta [9] found consistent differentials in milk and fat allocation between boys and girls, although caloric intake was roughly similar between the two groups.

> ...milk and fats are highly valued (and high-cost) foods of this society. They also have high nutritional quality. Among older children, boys are given a little more of each foodstuff, with the difference greatest in the case of fats.

Gender differentiation between children is less common, although Chen *et al.* [18] report favoring of male children over female children in Bangladesh. Age differentials have been found to favor children of both genders over adults in terms of caloric adequacy, especially in times of food scarcity [2, 36].

Gittelsohn [2] additionally found that household wealth and caste influence food allocation in Nepal. As expected, wealthier households do better nutritionally than poorer households. However, when controlled for economic effects, higher castes eat a decreased variety of foods and run a greater risk of certain nutrient deficiencies.

METHODOLOGY

Research setting

Research was conducted in Pahargaon* Village Development Committee (VDC)† in the Western hills of Nepal. The VDC is comprised of 15 villages (total population: approximately 4320). A total of 105 randomly selected households were studied, representing 767 individuals in six contiguous villages. Ethnically, the VDC is comprised of members of all four main caste groups (Brahmin, Chhetri, Vaisya and Shudra) plus significant numbers of Magar and Sunyasi households.

The mean household size for all castes in Pahargaon was 6.7 persons. Nuclear households are quite common as interpersonal problems frequently complicate relationships in extended or joint households.

A wide variety of income generating activities is employed by villagers, including subsistence agriculture, cash cropping (of ginger), manual labor, government service, business, politics, *jankri* (traditional healers), priests and artisan work. Women are only marginally involved in income-generating activities. Their province is primarily domestic, e.g. fetching water and firewood, maintaining the household, child rearing, and assisting their husbands with planting and harvesting the family fields. The elderly are usually accorded highly respected status, and are given the lightest household tasks. Small children are not expected to do much work, although informants often specified that little girls will often be required to "take care of their younger siblings, while boys study and play and eat". Children's household responsibilities increase as they grow older.

Foods available in the study area vary greatly with season, with the post-monsoon months (October–December) being the period of greatest abundance and the monsoon months (July–September) being the period of greatest scarcity. Most foods are produced by villagers on their own land; however supplementary food is purchased and sold in the villages or in nearby markets as well. Overall there is a large variety of foods, although even at times of abundance no more than a third of these foods are available.

In most households two main meals are consumed during the day, although snacking between meals by children is quite common. A "meal" is defined here as a gathering of household members for the purpose of food consumption, in which food is served by one household member to another (or to a guest). The term "meal" encompasses food selection, preparation, serving, timing, attendance, mood, ritual associations, interhousehold food-sharing and the treatment of guests.

Methods

Data were collected in five overlapping phases and included both qualitative and quantitative methodologies [2]. *Phase one* involved key informant interviews and participant observations as initial exploratory exercises. In *phase two* a demographic/economic survey was conducted on 115 households to collect individual demographic and anthropometric data within each household. *Phase three* involved unstructured pilot observations of meals in 20 non-study households. In *phase four*, dietary data was collected in 105 households, from May–August 1987. Dietary information was gathered on all household members, based on combined direct observation (centered around one of the two daily meals, but including snacking prior to the meal) and recall to obtain estimates of 24 hour intakes. The method used has been described in detail elsewhere [13]. Dietary data were collected on three to four occasions in each household on randomly selected days; there was no advance notification to households that their meals were to be observed/

*A pseudonym.
†Formerly termed *panchayat*.

recalled. Spot checks were also conducted on the households to determine time allocation patterns. In *phase five* key informant interviewing was reintroduced to clarify findings from the previous four phases. Focus-group interviews were conducted on a wide variety of topics and included groups of 2–6 people, usually of the same sex and caste group. Finally, structured pile sorts were conducted on subjects such as food classification.

Data analysis

Data analysis was performed using the SAS statistical analysis software package. Some of the outcome variables are presented in the form of Mean Nutrient Adequacy Ratios (MNAR), the percent of requirement met for a particular nutrient by the individual. Recommended safe levels of protein intake for individuals were calculated by multiplying the average safe protein allowance per kilogram of body weight (based on WHO/FAO recommendations for a particular age–sex group) by the average weight (kg) of a person in the corresponding age–sex group [37].

Individual energy requirements were calculated according to the following formula:

$$\textit{Energy Requirement (kcals)} = (BMR \times BMRC) + FPS$$

Basal Metabolic Rates (BMR) were calculated based on regression equations incorporating sex, age, and body weight [37]. Energy expenditure was expressed in terms of the BMR multiplied by a metabolic constant (BMRC), derived on an individual basis from observed daily activities, and including a factor for Female Physiological Status (FPS), when appropriate based on WHO/FAO recommendations [37].

Individual daily allowances for seven micronutrients (calcium, iron, beta-carotene, thiamin, riboflavin, niacin, vitamin C) were based on recommendations by the Indian Council of Medical Research [38].

As breastmilk intake was not quantified in this research, breastfeeding children (< 2 years) were eliminated from the analysis.

RESULTS AND DISCUSSION

Food belief systems

Khare [39] has described the "cultural economy" of the Hindu food system, which he sees as having both materialistic and emic-cultural components. Food selection, preparation, serving and consumption in Pahargaon is regulated by an interwoven set of beliefs and rules, including ways that food (and individuals) can become polluted, food classification systems, local explanatory models of illness (where food is perceived either as a causal agent or as a treatment), and normative patterns of favoring/disfavoring household members based on their age and gender. Many of these food beliefs have direct consequences for the diets of women [40].

Nichter [41] describes several food classification systems operating concurrently in a South Indian village. A version of the hot–cold food classification system is common throughout Nepal. Almost all foods can be assigned to one of three "valence" categories: hot, cold, or neutral. In Nepal, it is perceived that when an individual consumes a lot of food of a particular valence, illness can result, which can be corrected by consuming foods of the opposite valence [42]. The hot–cold valence of foods appears to be most important when persons are in a vulnerable state, such as pregnancy or the postpartum period.

In the present study, a mean "hot–cold score" was derived by interviewing 30 different local respondents. A mean valence ranking was calculated by assigning a score of −1 if the food was described as cold (*thundaa*), +1 if hot (*garmi*) and 0 if neutral (Table 1), and taking the average for all informants. Water buffalo milk (mean: + 1.00), *jwano* [caraway] soup (mean: + 1.00), millet alcohol (mean: + 1.00), chicken (mean: + 1.00), corn (mean: + 1.00) and soybeans (mean: + 0.98) were all listed as hot foods. Corn porridge (mean: − 1.00), cauliflower (mean: − 1.00), eggplant (mean: − 1.00) and pumpkin (mean: − 1.00) are among many foods considered cold. An analysis conducted on these data indicated a high level of consensus among the 30 respondents. Interestingly, there was more agreement by informants on what foods should be considered hot (+0.5 to + 1.0) or cold (−1.0 to −0.6), than on what foods should be considered neutral (−0.59 to + 0.49).

Many community members classify foods in terms of digestibility. In general, poorly cooked foods, fatty meats and many kinds of legumes, including soybeans, peanuts, peas and lentils are considered difficult to digest. These foods were said to make one sick, especially with diarrhea, vomiting or a cold. Digestibility is particularly important where the age of the consumer is concerned. Young children and the elderly are felt to require easily digestible foods (e.g. rice, nuts, sweets and yogurt) as do people in vulnerable phases of life or states (such as infancy or postpartum). Digestibility does not appear to be related to the hot–cold valence of the food, however.

Other less common systems of classification include taste and perceptions of healthiness. In general, Nepali foods have strong flavors: sweet, salty, sour and spicy. Foods that are not salty or are bitter are less popular. Bitter is perceived to be the taste of medicine, like *kaalo jiraa* (fenugreek). Particular care is take to ensure that small children do not consume spicy foods. VDC residents perceive certain foods as being beneficial to health. This notion of "healthiness" of a food is related in

Table 1. Mean hot–cold valence score

Cold (−1.00– −0.60)		Neutral (−0.59– +0.49)		Hot (+0.50– +1.00)	
Food	Score	Food	Score	Food	Score
[a]Corn (porridge/*roti*)	−1.00	Yams	−0.57	Breastmilk	+0.60
[a]Cauliflower	−1.00	*Ghee* (cow)	−0.54	Goat (milk)	+0.70
[a]Eggplant, taro	−1.00	Sweet potato	−0.49	Wild berries	+0.76
[a]Pumpkin	−1.00	Milk (cow)	−0.38	Wheat	+0.85
[a]Gourds, cucumber	−1.00	Lemon	−0.30	Peanut	+0.89
[a]Peaches	−1.00	Onions	−0.23	*Gundruk*	+0.89
[a]Chang (corn/rice)	−1.00	Broad beans	−0.18	Garlic	+0.91
[b]Tomatoes	−0.98	Bethe greens	−0.16	[b]Mustard oil	+0.96
[b]Yogurt (buffalo)	−0.98	Eggs	−0.13	[a]Tea (black/pepper)	+0.96
[b]Pork	−0.97	Coriander seed	+0.07	[b]Soybeans	+0.98
Apple, pears	−0.96	Dried beans	+0.07	[b]Corn (*khaja*)	+0.98
Green beans	−0.96	Blackeyed peas	+0.09	[b]Millet	+0.98
Banana	−0.93	Fern small	+0.12	[b]Red gram	+0.98
Black gram	−0.91	Sugarcane (black)	+0.15	[a]Fish	+1.00
Mustard leaf	−0.91	Mango	+0.34	[a]Chicken	+1.00
Guava, plum	−0.90	Orange	+0.40	[b]Milk (buff)	+1.00
Buff (meat)	−0.89	Pomegranate	+0.42	[a]*Raksi* (millet	+1.00
Okra, radish	−0.87	Lentil balls	+0.44	*Jwano* soup	+1.00
Yogurt (cow)	−0.83			[a]Natal	+1.00
Rice	−0.80				
Ghee (buff)	−0.74				

[a]Perfect agreement by respondents, 100% reported food as same valence.
[b]High agreement by respondents, 80—99% reported food as same valence.

part to a notion of the nutrient content of foods. The notion of certain foods as being "strengthening" or "weakening" is also common in the VDC, and relates to the concept of health-promoting foods.

Food consumption through the life span

The food belief systems described above are applied to individuals differentially over their life spans. The data presented in this section are primarily based on reported beliefs and behaviors, though much of the information was verified through direct observation [13].

Some dietary rules exist for breastfeeding women. In particular, if the breastfeeding child is in a vulnerable state, dietary restrictions may be followed by the mother, as it is perceived that qualities associated with specific foods are passed on to the child through breastmilk. The primary proscription appears to be avoidance of certain foods when the infant is ill so that qualities of "coldness" and "indigestion" will not be passed along. An exception are fresh green leafy vegetables which are avoided continuously during lactation, as they are perceived as "cold" and are thought to cause arthritis, swelling and other illnesses in both the mother and infant. Instead, lactating women dry green leafy vegetables before eating them (eliminating the majority of water-soluble vitamins).

Through infancy and childhood, children of both genders are often shown favored treatment in terms of food, receiving special foods when available. On the other hand, food avoidances during illness appear to be most stringently observed for small children. No differences were observed in the feeding of young boys compared with girls.

Sometime between the second and fourth year of life, Pahargaon children begin to consume the regular adult diet, although they may receive less heavily spiced food than their parents. As children move into puberty and adolescence, they begin to assume adult roles and responsibilities. Boys start to work in the fields and/or attend school. Girls begin to take up domestic responsibilities, including child care, water and wood fetching, and herding. Older daughters begin to be served last with their mothers in some households. While only 19% of mothers reported differential feeding by the gender of the child at adolescence, our observations indicate that this practice is more pervasive. Reasons emphasized that boys needed to be strong (for work) and that they needed more food because they would be expected to take care of the family in the future.

In adulthood, the distinctions made between Pahargaon males and females emerge in full force, with pronounced favoring of males in food distribution. These patterns are expressed in the order in which persons are served, in amounts of food given, and in the preferential allocation of special, high status foods, resulting in a somewhat greater variety of foods for males. Early adulthood appears to be a time of particular stress for rural Nepali women. A woman marries and moves to her husband's household, where she has very low status. She is expected to do a great deal of the hard physical work, produce children and not complain about her state. In Pahargaon, while usually permitted to eat their fill of staple foods, junior adult females receive subtle discouragement in the consumption of special foods (e.g. animal products, special fried foods). They are not encouraged to eat second helpings of these foods. When new daughters-in-law begin to fulfill

their reproductive role through the production of offspring, their overall status increases.

Generally, pregnancy is not seen as a time for special food restrictions or prescriptions. Mothers were asked what foods they consumed specially during their previous pregnancy; 38.6% stated that they consumed no special foods during pregnancy, and merely ate the regular family diet. Five percent of mothers interviewed said they consumed certain foods preferentially during pregnancy. Animal products, especially meat and dairy products were the most frequently mentioned food items to be eaten preferentially. The most common reason women gave was due to "craving", not to any perceived quality of the food.

During the postpartum period, when the mother and infant are considered to be especially vulnerable to cold, new mothers will avoid working in the rain, doing laundry and planting rice, all activities associated with "cold" water. There is agreement among respondents that hot foods *should* be consumed during this period. Limited observations on postpartum women indicate that certain hot foods like *jwano* soup, chicken, fish and millet *roti* are indeed preferentially consumed. Many of these foods are good sources of animal protein and would seem to be advantageous to Pahargaon women. However, the most commonly mentioned avoided foods are *also* hot foods; for example soybeans, wheat bread, peanuts and corn porridge, which are believed to cause indigestion in mothers and infants, illustrating how food choices are the result of multiple overlapping belief systems.

As Pahargaon men move into adulthood, their status increases. They assume primary responsibility for decision-making about the household, though adult women may have an important advisory role. The higher status of adult males is recognized in many ways, including household food behavior. In general, the adult male is considered the least vulnerable of any state; thus, there are few concerns about avoiding foods that affect those in vulnerable states, unless he becomes ill.

Elderly persons, both male and female, are seen as being in a vulnerable state, requiring more strength-giving foods. If a household member is very elderly, foods will be prepared that are easier to consume. In general, "cold" foods are not given to the elderly for fear of causing illness.

Effects on dietary intake and adequacy

This section describes patterns of intrahousehold variation in dietary intake and nutrient adequacy in Pahargaon VDC. As dietary intake data were collected from May–August 1987, we will not be able to present seasonal differences. The dietary assessment period took place during the pre-monsoon and monsoon seasons. This period is characterized by overall scarcity of most foods, except domestic and wild fruits, which are highly available.

Table 2 shows how differences in gender and transitory states are played out in terms of the consumption of food types during household meals. Numbers in the table represent the percent of persons who ate a food from a particular group during an observed meal. Chi-square tests were employed to detect whether the differences observed between adolescent males, adult males, adolescent females, non-pregnant and non-lactating females and lactating females were due to chance alone. The number of meals observed with menstruating, pregnant and postpartum women were too small to include in the test, but averages are presented in the table for comparative purposes.

For staple grains (rice, wheat *roti*), no significant differences were observed between any of the five groups; almost everyone received at least one kind of staple food during a meal. Nor were any significant differences observed in the number of persons consuming of *daal* (lentil soup), meat/eggs and pickles.

Adult males were significantly more likely to consume vegetables than adolescent males ($\chi^2 = 7.068$, $p = 0.008$), and lactating females ($\chi^2 = 4.276$, $p = 0.039$). They are more likely to receive milk and milk products than adolescent males ($\chi^2 = 2.726$, $p = 0.099$), adolescent females ($\chi^2 = 3.309$, $p = 0.069$) and lactating females ($\chi^2 = 10.095$, $p = 0.001$). These findings reinforce their position as the preferred recipient of food during mealtimes.

On the other hand, adolescent males were significantly more likely to consume fruits during a meal than either adult males ($\chi^2 = 4.673$, $p = 0.031$) or

Table 2. Gender/physiological status-based differences in % persons receiving at least one item from food category (during a meal)

				Adult females (18—50 years)				
Food group	Adolescent males (10–18 yr) (n = 141)	Adult males (18–50 yr) (n = 207)	Adolescent females (10–18 yr) (n = 163)	Non-preg. Non-lact. (n = 143)	Lactating (n = 186)	Menstruating (n = 16)	Pregnant (n = 20)	Postpartum (n = 20)
Staple grain	96.3	95.8	97.0	99.3	97.3	93.8	100	90.0
Daal	28.7	31.1	30.7	25.9	29.6	31.3	15.0	30.0
Vegetable (TK)	46.3	60.9	53.6	53.2	50.5	62.5	80.0	30.0
Saag (DGLV)	7.4	7.6	12.7	12.6	7.0	12.5	5.0	10.0
Meat, eggs	12.5	12.7	9.0	14.7	9.7	0	10.0	30.0
Milk/*ghee*	28.7	37.3	30.7	28.0	22.6	18.8	30.0	25.0
Achar/pickles	14.7	17.5	16.3	15.4	15.1	12.5	15.0	5.0
Fruit	22.1	13.2	19.3	8.4	16.7	25	40.0	10.0

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adult females (χ^2 = 10.182, p = 0.001). Adolescent females were more likely to consume fruit than adult females (χ^2 = 7.454, p = 0.06). These results relate to the unique position of fruits as a food category. Fruits were relatively abundant during the observation period (May–August), and many were gathered wild by adolescents and small children, consumed during the meal observation period, but not as part of the prepared meal.

Lactating women consume less DGLV (dark green leafy vegetables) (χ^2 = 3.222, p = 0.073) and milk (χ^2 = 2.991, p = 0.084) than adolescent females, and less DGLV (χ^2 = 2.969, p = 0.085) than adult females. The results concerning DGLVs would appear linked to food proscriptions for lactating women reported earlier. On the other hand, lactating women appear to be eating more fruit than non-lactating adult females (χ^2 = 4.873, p = 0.027).

While the sample size was too low to calculate chi-square statistics for women in different phase states (menstruating, pregnant, postpartum), overall differences in consumption of food types were consistent with expressed beliefs about food proscriptions. These food consumption patterns have consequences for the adequacy of the local diet. In general, women are less likely than men to meet their requirements for kilocalories, with significant differences observed between males and females 3–6 years, 10–14 years, and 25–49 years (Fig. 2). While able to eat their fill of staple foods, it appears that these less energy-dense foods alone are insufficient to meet women's caloric needs in many cases, particularly those women with increased needs due to pregnancy and lactation. Interestingly, children are more likely to meet their energy requirements than adults of both sexes.

The intake of beta carotene shows a similar pattern, with significant differences in mean intake relative to requirement between adult (>25 years) men and women. Similar age-related variation, with children doing better than adults, was found for beta-carotene as well (Fig. 3). Children are frequently "channeled" special foods in short supply, like milk, sweets and fruits.

For vitamin C, significant differences were observed only between adult men and women (Fig. 4). Again, for this nutrient, children tend to do better than adults. Fruits were the main source of vitamin C in the diet during the dietary assessment period. As most fruits are eaten as snacks, their frequency tends to be underreported in Table 2.

Figures 2–4 indicate very low intakes (relative to requirements) of calories and micronutrients for reproductive-aged women. This appears linked largely to food restrictions for pregnant and lactating women (included in the figures), and to increased nutrient requirements considered in calculating their energy and micronutrient requirements.

Determinants of dietary adequacy

In order to examine the effect of gender and age of the individual in the context of other household and individual-level variables, a series of multiple linear regressions were calculated for all nutrients, incorporating a variety of other variables (Table 3). The other variables tested in the model included "respect" (a measure of relative household status based on a ranking of kin types assigned by key informants), "overall wealth" (based on land ownership, material possessions, housing quality, and animal ownership), "meal size" (mean number of people present during a household's meal),

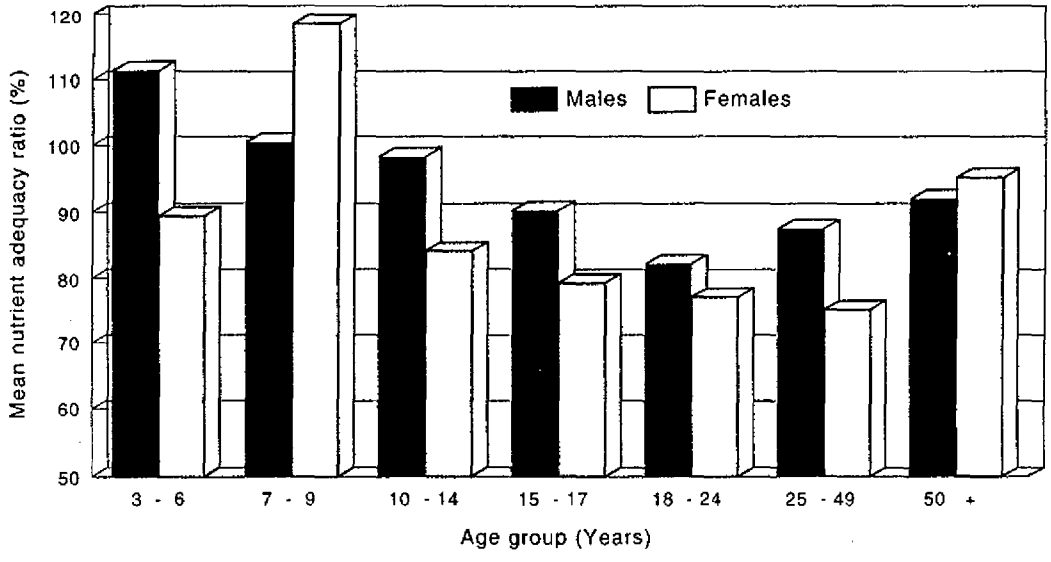

Fig. 2. Intake of KCals in Nepal: varaition by sex.

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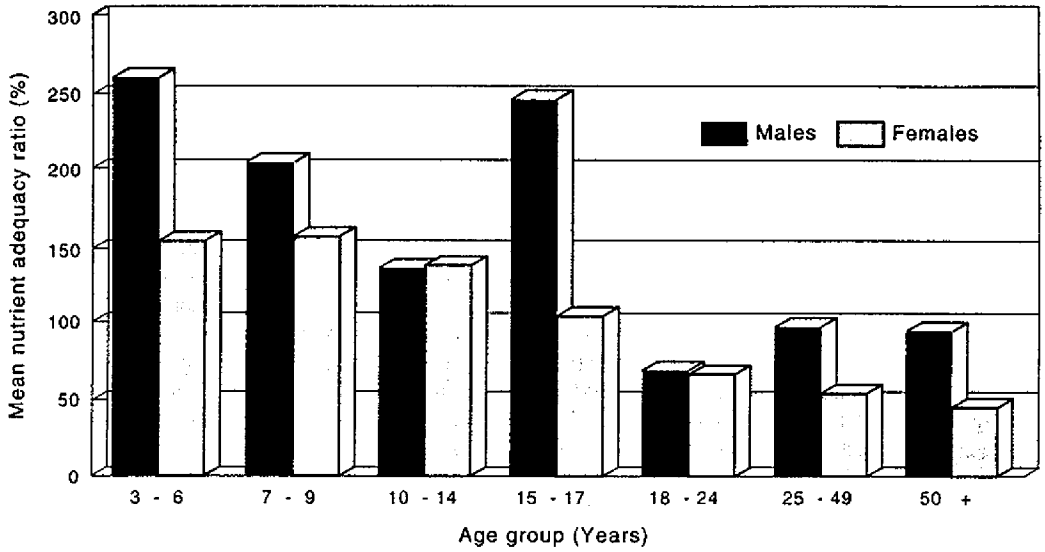

Fig. 3. Intake of beta carotene in Nepal: variation by sex.

"education" (in years), "caste", "meal phase" (proportion of regular household members present during a meal) and "household type" (higher numbers meaning more generations, presence of extended family and non-family members, etc.).

R-squares for these regressions were low, accounting for 5–15% of the variance. Many variables were significant predictors for kilocalorie MNAR scores. Age was the strongest predictor, with young children most likely to have high scores, as well as overall household wealth. Higher Kcal MNAR scores were also associated with being male and from a higher respect grouping. Variables that seemed to be associated with increased intakes of other micronutrients included small meal size (for kcals and protein), higher education (for iron, vitamin C and calcium), higher caste status (for protein and calcium) and smaller, less complex households (for beta carotene and vitamin C). Overall, male sex and age (although the direction for age differs by nutrient) are the most important predictors for adequacy of nutrient intake in these models.

Caloric sufficiency and micronutrient sufficiency

Given that men appear to have larger intakes of nutrients than women (relative to requirements) and that patterns of allocation appear to favor men's intake over that of women for particular foods (especially non-staple luxury foods), it seems a clear possibility that in the context of rural Nepal there is a lack of relationship between energy intake, primarily derived from staple diet foods (rice, wheat

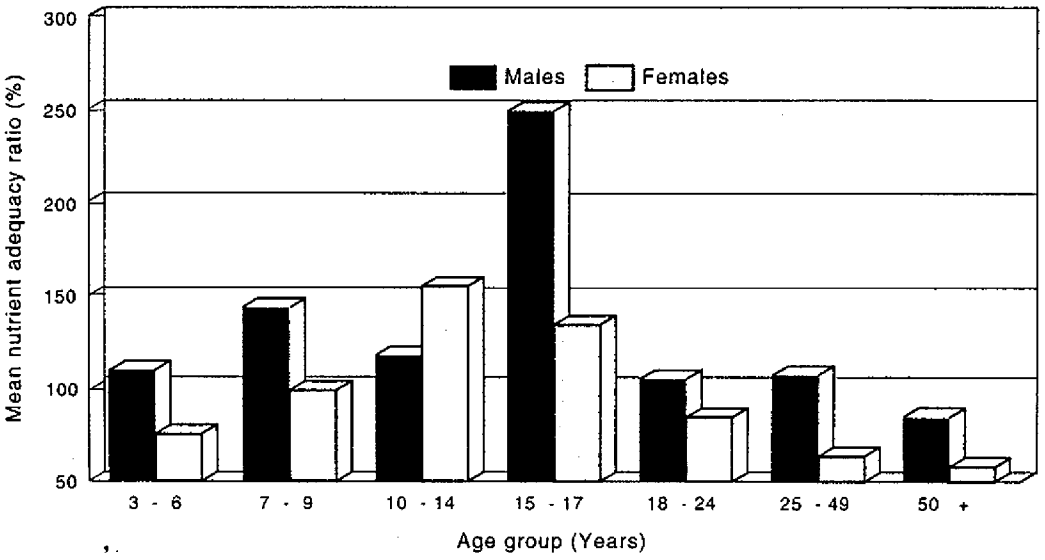

Fig. 4. Intake of vitamin C in Nepal: variation by sex.

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Table 3. Summary of multiple regression results: individual and household-level determinants of MNAR scores for key nutrients

	Nutrient											
	KCals (R^2 = 0.12)		Protein (R^2 = 0.13)		Beta carotene (R^2 = 0.09)		Iron (R^2 = 0.08)		Vitamin C (R^2 = 0.05)		Calcium (R^2 = 0.14)	
Variable	Beta	$p < T$	Beta	$p < T$	Beta	$p < T$	Beta	$p < T$	Beta	$p < T$	Beta	$p < T$
Age	−0.245	****	−0.198	****	0.157	****	0.065	*		NS	0.136	****
Sex (− = male)	−0.065	*	−0.080	**	−0.132	****	−0.171	****	−0.063	*		NS
Respect	0.128	***		NS		NS		NS	−0.079	**	0.110	**
Overall wealth	0.183	****		NS	0.074	**		NS		NS	0.211	****
Meal size	−0.083	**	−0.085	**		NS		NS		NS		NS
Education		NS	−0.125	****		NS	0.139	****	0.127	****	0.106	****
Caste (− = higher)		NS	−0.062	*		NS		NS		NS	−0.107	****
Meal phase		NS		NS		NS	−0.063	*		NS	−0.056	*
Household type		NS		NS	−0.083	**		NS	−0.133	****		NS

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
****$p < 0.0001$.

and corn), and micronutrient intake, derived largely from "luxury" or non-staple foods (meat, vegetables, dairy products, beans and pickles).

Energy and protein intakes are correlated across all age and sex groups. However, energy intakes are not correlated with beta-carotene, iron and vitamin C intakes for older individuals and, in particular, for adult females (Table 4). In general, women are more likely than men to have micronutrient intakes that are *not* correlated with energy intakes. Even among other age and sex groups, correlations between energy and micronutrients are low. In this setting, then, it appears that a diet high (or low) in energy is not necessarily high (or low) in other micronutrients.

What are the implications of these findings for overall dietary adequacy? Table 5 shows the percent of male and female adults with MNAR scores below 100% for different nutrients. The number of adults with less than 100% ranges from nine (protein) to 159 (beta carotene). The data have been disaggregated so that the percent of men and of women who are *calorically sufficient* (KCal MNAR scores > 100%) can be compared to those who are *calorically insufficient* (KCal MNAR scores < 100%). In both men and women, high percentages of individuals have both low intakes of micronutrients and low intakes of calories, ranging from 4% of adult males for iron to 52% of adult females for beta carotene. For all nutrients, women are more likely than men to have both energy intakes and micronutrient intakes less than 100% of the MNAR.

Significant numbers of men and women are calorically sufficient and micronutrient insufficient, ranging from a low of 2% of adult males for iron and calcium to 32% of adult males for beta-carotene. Larger percentages of adult women than men are calorically sufficient, but have insufficient intakes for micronutrients for iron, vitamin C and calcium. Adult men are more likely to be calorically sufficient, but have sufficient intakes of beta carotene.

CONCLUSIONS

Sufficient caloric intake does not assure sufficient micronutrient intake in this setting. From our data,

Table 4. Correlations between mean daily energy intake and intake of other nutrients by age and sex

	Age group									
	Children (2–10 years)		Adolescents (10.1–18 years)		Young adults (18.1–30 years)		Adults (30–50 years)		Older adults (50+ years)	
Nutrient	Male (n = 84)	Female a(n = 75)	Male (n = 48)	Female (n = 53)	Male (n = 32)	Female (n = 65)	Male (n = 51)	Female (n = 61)	Male (n = 19)	Female (n = 16)
Protein	0.761 ****	0.571 ****	0.612 ****	0.573 ****	0.767 ****	0.673 ****	0.658 ****	0.612 ****	0.847 ****	0.752 ***
Beta-carotene	0.306 **	0.383 ***	0.338 *	0.100 NS	0.420 *	0.192 NS	0.147 NS	0.3260 *	0.517 *	0.160 NS
Iron	0.503 ****	0.636 ****	0.469 ***	0.220 NS	0.338 NS	0.306 *	0.320 *	0.390 **	0.670 **	0.323 NS
Vitamin C	0.391 ***	0.230 *	0.286 *	0.160 NS	0.242 NS	0.137 NS	0.115 NS	0.223 NS	0.441 NS	0.302 NS
Calcium	0.514 ****	0.466 ****	0.608 ****	0.369 **	0.545 **	0.427 ***	0.419 **	0.534 ****	0.666 **	0.369 NS

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
****$p < 0.0001$.

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Table 5. Micronutrient sufficiency related to energy sufficiency (adults 18–50 years only; n = 85 males, 128 females)

Nutrient (MNAR < 100% only)	Calorically insufficient MNAR KCals < 100%		Calorically sufficient MNAR KCals > 100%	
	Male	Female	Male	Female
Protein (n = 9)	4% (3)	5% (6)	0% (0)	0% (0)
Beta-carotene (n = 159)	38% (32)	52% (67)	32% (27)	26% (33)
Iron (n = 30)	4% (3)	16% (20)	2% (2)	4% (5)
Vitamin C (n = 123)	28% (24)	43% (55)	16% (14)	23% (30)
Calcium (n = 55)	14% (12)	26% (33)	2% (2)	6% (8)

it appears that while there is a positive relationship between caloric intake and the intake of some micronutrients at the population level, this pattern does not hold true for certain subgroups within the population, in particular adult women and adolescent girls. In the rural Nepali setting, these subgroups appear to be at a particular risk for diets deficient in vitamin A and vitamin C.

In Pahargaon, food beliefs and practices appear to place individuals at risk for nutritional deficiencies and may account (at least partially) for the low correlation between energy and micronutrient intakes. Our dietary information, comprised of both recalled intake and direct observation of meals, shows that while there is variation in individual application of these cultural rules, dietary proscriptions and prescriptions directly influence actual food consumption behavior. Gender-based differences in access to certain foods appear linked, in part, to specific food beliefs and practices that reduce women's consumption of micronutrient-rich foods, such as dietary restrictions during menstruation, pregnancy and lactation [43]. Overlapping with these beliefs and practices, an overall pattern of discrimination against women in the intrahousehold allocation of food is evident in the study communities. While staple food items (i.e. rice, lentil soup, bread, etc.) are distributed fairly equally, side dishes usually containing more micronutrients (i.e. vegetables, meat, yogurt, *ghee*, etc.) are often preferentially allocated to valued household members, including adult males and small children (of both sexes).

To what extent can women's poorer nutrient intakes be attributed to culture-based food proscriptions versus overt discrimination? Differences observed between lactating and non-lactating women suggest that food proscriptions do affect dietary choices independent of discrimination based on gender. Unfortunately, the small number of women sampled who were menstruating, pregnant or postpartum do not permit statistical comparisons with these groups. Such knowledge would be crucial for planning nutrition education strategies to improve the lot of Nepali women, but must await future studies.

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43. Interestingly, the postpartum period involves preferential intake of "hot" (high protein) foods, although there are some "hot" food proscriptions as well.

See corresponding article on page 160.

What do indicators indicate?[1,2]

Jean-Pierre Habicht and Rebecca J Stoltzfus

Selection of the best indicators is essential for scientific progress and for implementation of programs. de Pee et al (1), in this issue, show that different investigators rank indicators differently. One explanation is that different procedures reveal different aspects of an indicator. It is important that the aspects revealed are relevant to the indicator's uses. Certain principles are at hand that permit a more systematic approach to reveal these relevant aspects (2, 3).

An indicator is a measurable variable that is supposed to indicate some underlying reality that is more difficult or impossible to measure. One cannot evaluate an indicator without first specifying that underlying reality (4). Serum retinol is an indicator of vitamin A status—the underlying reality. However, in practice our definitions of vitamin A status may vary with the particular question being addressed. Vitamin A status might mean total body stores of vitamin A, vitamin A uptake by one or more target tissues, or vitamin A balance. In public health nutrition the underlying realities of interest are usually not nutrient status per se. When we use indicators to make public health decisions, the correct decision concerns the underlying reality. The decision of interest might concern the number of children at risk of death from vitamin A deficiency and its consequences, or whether a group of women will benefit from vitamin A supplementation, or whether a particular vitamin A intervention had the intended effect. The performance of an indicator will depend on the underlying reality that it is supposed to indicate. This issue is inadequately addressed among nutrition scientists and public health nutritionists.

Once the context of the evaluation is specified, to evaluate an indicator's performance the indicator is compared with a gold standard, which is an approximation of the underlying reality. For an indicator to be useful it must either be summarized for a population by a mean or a rate, or it must be dichotomized by a cutoff value or trigger level, respectively, for classifying individuals or populations. Ranking the performance of indicators requires presentation of the indicators' sensitivity-specificity characteristics over their full range (5) and not only at a single cutoff to be able to test the ranks statistically with adequate power (6). Considerations of these issues (7) and methods to address them (8) can be found elsewhere.

The ideal gold standard for vitamin A indicators is often stated to be liver vitamin A stores (1). In practical situations, this is almost never the case. The fundamental problem lies with the definition of reality, not with its approximation (which is also problematic). The reality must relate to the decision that will be made on the basis of the indicator's measurement. A full review exceeds the space available in this editorial, but a few examples illustrate the approach.

One set of decisions relates to the consequences of vitamin A deficiency. First one needs to decide that vitamin A deficiency is truly an important determinant of the supposed consequences. Then one must decide whether to treat or prevent the consequences through vitamin A interventions, and finally how to monitor the effect of those interventions. An example would be to use the indicators described in the first section of de Pee et al's article to screen mothers for a vitamin A intervention to prevent mortality in their infants. The appropriate gold standard for this decision would be the prevented deaths, and the indicators would be ranked on how well they predicted these prevented deaths. This type of analysis has been done for anthropometric indexes and risk of child death, but not yet for deaths preventable by interventions (2).

A second use of the indicators in the first part of de Pee et al's article might be to screen mothers for maternal interventions to improve infant vitamin A intake from breast milk. The best indicator would best predict the rise in breast-milk vitamin A from the intervention. Thus, the gold standard is the rise in breast-milk vitamin A.

A third use of indicators is to evaluate interventions. This is the subject of the second part of de Pee et al's article. It is important to differentiate indicators of responsiveness to interventions from those indicators that predict benefit from an intervention. The best indicator for these tasks may be different. For example, in an anemic population low serum ferritin may perform well to predict benefit (cure of anemia) from an iron intervention. But ferritin concentrations might not be highly responsive to the intervention because the absorbed iron will be utilized for hemoglobin synthesis, not stored.

The performance of an indicator of response may be influenced by the sort of intervention given, the statistical treatment of the variable, and the range of status of the population. Each of these is illustrated by de Pee et al's study. First, the gold standard used by de Pee et al was the response to a low-dose, five-times-weekly intervention with carotenoids, whereas the

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intervention to which the results were compared (5) was a single high dose of vitamin A (9). This difference may explain the low responsiveness of milk vitamin A in de Pee et al's study. Second, and more important, de Pee et al point out that they used the pre- to postintervention increments in indicator values within individuals, whereas the other analysis used postintervention values. Homeostatically controlled indicators, such as serum retinol, are usually much less variable within an individual than they are across individuals. Thus, they will require much smaller sample sizes to identify differences between groups of women if increments are used rather than absolute values. The opposite is true for nonhomeostatically controlled indicators such as vitamin A in milk. For instance the sample sizes needed to detect differences at 3 mo between postintervention values of groups of women receiving a single high dose of vitamin A or placebo was 148 for serum retinol, 60 for breast-milk vitamin A, and 35 for breast-milk vitamin A as a ratio to fat (5). The respective sample sizes needed for the same comparisons but using the pre- to postintervention increments were 47%, 62%, and 143% as large (RJ Stoltzfus, unpublished observations, 1997).

Third, because of the homeostatic control, serum retinol is less responsive at higher rather than lower levels of vitamin A nutriture. Theoretically, breast-milk vitamin A concentrations will be responsive to vitamin A nutriture at higher concentrations, and thus will tend to respond better to vitamin A interventions than will serum retinol in populations with better vitamin A status. There is some evidence for this in cows (10).

These examples of decisions related to consequences need to be expanded and complemented by examples related to other kinds of decisions, such as those related to nutritional equity or to the search for hypothesized benefits of vitamin A nutriture higher than those necessary to prevent death. For some of these uses liver vitamin A stores might be a useful gold standard, but the case needs to be made that this is so, and if so, that it is practically feasible.

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Linear Growth Retardation in Zanzibari School Children[1,2,3]

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ABSTRACT This paper describes the longitudinal changes in height and weight of children in school grades 1–3 on Pemba Island, Zanzibar, a poor rural population in which parasitic infections and anemia are highly prevalent. Heights and weights of children were measured at base line, and 6 and 12 mo later, and were compared with U.S. reference data. At base line, the prevalence of height-for-age Z-score < −2 rose from 14% in 7-y-old children to 83% in 13-y-old children. Prevalence of weight-for-age Z-score < −2 in children < 10 y was ~10% or less. Median 6-mo height increments for Pembian boys were around the 5th percentile at age 8 and around the 10th percentile from age 9 to 13 y. Height increments for girls improved from below the 25th percentile to above the median in this age range. Based on the longitudinal yearly gains observed, boys accumulate a height deficit of 11.9 cm and girls 8.5 cm, relative to the reference population. In multivariate analyses, a small part of the variability in growth increments was explained by ascariasis and anemia (for weight gain) and schistosomiasis (for height gain). A review of other growth data from rural African Bantu populations provides supporting evidence that stunting occurs in older as well as younger children. It has been controversial whether school-based health and nutrition interventions could induce catch-up growth in already stunted children. Our results suggest that appropriate interventions might actually prevent stunting in late childhood. J. Nutr. 127: 1099–1105, 1997.

KEY WORDS: • *Africa* • *school children* • *growth* • *stunting*

Improving the health of school children is emerging as a policy priority in international health. The recent World Bank Development Report, *Investing in Health*, recommended school-based health services as a top priority for public health action in developing countries after concluding that they are among the most cost-effective activities for reducing the burden of disease in developing countries. Proposed models for school-based health services typically include distribution of anthelminthic medications and micronutrient supplements, along with health education (Savioli et al. 1992, World Bank 1993).

Although it is likely that school-based interventions may prevent or cure micronutrient deficiencies in school children, it remains controversial whether these interventions could improve growth, particularly linear growth. Because linear growth retardation is believed to occur mainly in early childhood (Martorell et al. 1994, WHO Expert Committee 1995), the question has focused on whether stunted children can exhibit catch-up growth if their environment is improved in later childhood. Adopted children whose environment changes from one of poverty to one of adequacy can attain a normal adult height (Proos et al. 1991), and some authors have concluded that catch-up growth may occur even when children remain in their impoverished environment (Kulin et al. 1982). However, others maintain that catch-up growth does not occur, except in unusual populations in which maturation is markedly delayed (Martorell et al. 1979 and 1994).

Schools are usually the most efficient channel for delivering health interventions to this age group, and school-based surveys are most often used to assess need for different interventions. Although a survey of height and weight of school children is relatively simple to implement, the data may easily be misinterpreted. Shortness-for-age (being stunted) is common among school children in developing countries. However, it is believed to reflect a process that almost always happened in early childhood (Martorell et al. 1994). If linear growth failure (stunting) is not happening during the school years, then it cannot be prevented by school-based interventions (although it might be overcome by catch-up growth). In addition, there are many pitfalls when using cross-sectional data from school children in a search for age effects. Nine-year olds may be more stunted than 7-y olds because they were more malnourished as young children, not because they are growing poorly as school children. Also, school enrollment and drop-out patterns may differ in stunted and nonstunted children, creating a spurious picture of stunting by age among children who attend school.

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As part of an evaluation of a school-based deworming program, we monitored the longitudinal changes in height and weight of Zanzibari school children over a 1-y period. Data from the nonprogram schools enabled us to examine both the cross-sectional and longitudinal patterns of growth in primary school children in the absence of intervention. As we expected, the cross-sectional and longitudinal pictures differ in important ways. Contrary to our expectation, we found that these children are experiencing stunting of an important magnitude during the school years. These results are relevant for the planning and evaluation of school health programs in East Africa and perhaps other regions.

SUBJECTS AND METHODS

Study population. This program evaluation was conducted on Pemba Island, the smaller of the two islands of Zanzibar, a part of the United Republic of Tanzania. Pemba Island lies 40 km off the East African coast, 5° south of the equator. The island is divided from north to south into four districts of approximately equal size. The population is predominantly rural but extremely dense, about 330 people/km^2. The economy is devoted primarily to subsistence agriculture and cloves, an exported cash crop, with practically no tourism. There are two rainy periods of the year, the short rains around November and the long rains from mid-March to mid-May. There is little irrigation, making patterns of work and harvests markedly seasonal. The primary staple foods are cassava and rice, the latter being more desired but less affordable. These foods are eaten with sauces of vegetables and small fish. Larger fish and meat are luxury items, and there are pronounced seasonal shortages in vegetables and fish. Like other parts of coastal East Africa, Pemba Island is characterized by intense transmission of *Plasmodium falciparum* malaria, *Schistosoma haematobium* and geohelminths.

The present analyses were conducted using data collected in four schools that were assigned as a control group for an evaluation of a school-based deworming program. To form this control group, one school was randomly selected from the public primary schools in each of the four districts of the island and morning classes of grades 1–4 were selected. The base-line survey was conducted in March–May, 1994, and anthropometric measurements were conducted at base line, and 6 and 12 mo later. In the control schools, 1375 children were enrolled in the selected classes according to the school records, and 1261 (91.7%) of those children participated in the base-line survey. Children who were surveyed again 6 mo later (86.7% of those at base line) and who had sex and age data at base line were used in the present analyses; this sample numbered 1090 children, 86.4% of the base-line cohort. Of these children, 1072 (98.3%) had anthropometric data at base line and 6 mo, and 978 (89.8%) had complete data at base line, 6 and 12 mo.

The study protocol was reviewed and approved by the institutional review boards of The Johns Hopkins University, the Ministry of Health of Zanzibar and the World Health Organization.

Assessment of nutrition and health status. Two anthropometrists were trained and standardized at the start of the study, but >95% of height measurements at all surveys were taken by one of the anthropometrists, who used the same equipment at each survey time. Children were weighed and measured in light clothing without shoes. Weight was measured to the nearest 0.1 kg using a battery-powered digital scale (Seca, Colombia, MD), and height was measured to the nearest 0.1 cm using a wooden stadiometer (Shorr Productions, Olney, MD). Age was calculated from the birth date on school records, which are taken from birth certificates, a requirement for school registration. Occasionally, however, the school had no record of age, and the child's self-reported age was used.

Hemoglobin concentration was determined by use of the HemoCue (HemoCue AB, Angelhom, Sweden). Parasitologic methods are described in detail elsewhere (Stoltzfus et al. 1997). Briefly, thick and thin blood smears were fixed and stained with giemsa, and malaria parasites were counted against leukocytes, using standard methods. Malaria species was also identified. All infections were *P. falciparum*; in <5% of slides, *P. malariae* was also identified. Prevalence and intensity of helminth infections were determined using the Kato-Katz method (World Health Organization 1994). *S. haematobium* infections were determined indirectly by testing for microhematuria with Hemastix test strips (Ames Laboratories, Elkhart, IN); grade ++, +++ or the presence of visible hematuria was considered a positive test. This procedure screens for *S. haematobium* infection with 69% sensitivity and 86% specificity (Savioli et al. 1990). Any child with visual hematuria was treated with praziquantel.

Data analysis. Height-for-age and weight-for-height Z-scores were calculated using EpiInfo (Centers for Disease Control, Atlanta, GA). Stunting was defined as height-for-age Z-score <−2. Wasting as weight-for-height Z-score <−2. We used this indicator only for children <10 y old because it is not recommended beyond this age (WHO Expert Committee 1995).

Six-month height and weight increments were calculated as the difference in measurements between surveys, adjusted to 6-mo differences according to the actual number of days between surveys, which were approximately but not exactly 6 mo apart. These increments were compared with the incremental growth tables of Baumgartner et al. (1986), based on the growth of white children from Ohio (the Fels Longitudinal Study). For this purpose, an age variable in 6-mo increments was created, in which children aged 6.75–7.24 y at base line were categorized as age 7 y, children 7.25–7.74 y as 7.5 y, and

TABLE 1

Zanzibari school children: study cohort at base line

	Boys	Girls
	n (%)	
All	549 (50.4)	541 (49.6)
Age (y)[1]		
<6.75	2 (0.4)	2 (0.4)
7	6 (1.1)	8 (1.5)
7.5	13 (2.4)	6 (1.1)
8	24 (4.4)	20 (3.7)
8.5	29 (5.3)	21 (3.9)
9	54 (9.9)	59 (10.9)
9.5	44 (8.0)	60 (11.1)
10	89 (16.2)	107 (19.8)
10.5	45 (8.2)	28 (5.2)
11	86 (15.7)	93 (17.2)
11.5	28 (5.1)	34 (6.3)
12	46 (8.4)	60 (11.1)
12.5	22 (4.0)	14 (2.6)
13	28 (5.1)	17 (3.1)
>13.25	33 (6.0)	12 (2.2)
School grade		
1	225 (41.0)	201 (37.2)
2	220 (40.1)	229 (42.3)
3	104 (18.9)	111 (20.5)
Parasitic infections[2]		
Malaria	310 (57.0)	301 (56.3)
Ascariasis	393 (75.0)	378 (72.1)
Trichuriasis	492 (93.9)	503 (96.0)
Hookworms	485 (92.6)	474 (90.5)
Schistosomiasis[3]		
Microhematuria	166 (30.5)	143 (26.7)
Visual hematuria	17 (3.1)	13 (2.4)
Anemia[4]		
Hb <110 g/L	355 (64.7)	319 (59.0)
Hb <70 g/L	23 (4.2)	17 (3.1)

[1] Except for oldest and youngest groups, age refers to midpoint of age group, e.g., 7 includes children from 6.75 to 7.24 y, 7.5 includes children from 7.25 to 7.74, and so on.

[2] Numbers for parasitic infection and anemia do not match total number of children because data were occasionally missing.

[3] Hematuria was used as an indicator of urinary schistosomiasis as described in Subjects and Methods. Hematuria categories are mutually exclusive.

[4] Hb, hemoglobin.

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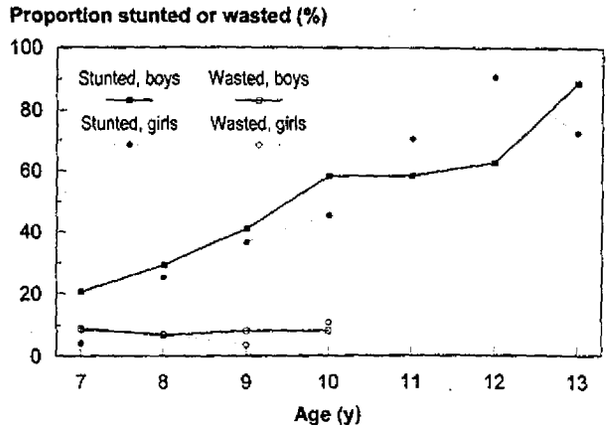

FIGURE 1 Prevalence of stunting and wasting in Zanzibari school children by age and sex. Data are from base-line assessments. For numbers of children, see Table 1; children plotted at age 7 y include children 7.0 and 7.5 y, and so on.

so on. In the tables and figures that follow, our age groupings represent the age at the start of each growth interval. This is in contrast to the tables of Baumgartner et al. (1986), in which the tabulated age is at the end of the growth interval.

To illustrate the magnitude of the height and weight deficits at different ages in this population, we calculated the difference between each child's height or weight and the median height or weight of the WHO growth reference population (World Health Organization 1983). The difference in the height or weight deficit at base line and at 12 mo represents the height or weight deficit accrued by a child in a 1-y period relative to the growth reference. The average annual height or weight change relative to the reference was calculated for boys and girls, along with 95% confidence limits (Snedecor and Cochran, 1980).

To test potential predictors of variation in growth rate, we used multiple linear regression, with the base-line to 6-mo height or weight increment as the dependent variable. In both models, we first adjusted for the variation due to age, sex and school, then tested additional predictor variables and retained those with significance levels below 0.10. Interactions between predictor variables were also tested and considered significant at $P < 0.20$. Data analyses were conducted using Systat statistical software (SYSTAT, Evanston, IL).

RESULTS

The study sample was comprised of almost equal numbers of boys and girls (Table 1). Although all children were in grades 1–3, the age range of the children was wide, because late school entrance is common in Zanzibar. The prevalence of anemia in these school children was very high; this is described in detail elsewhere (Stoltzfus et al. 1997). Over half of children had circulating malaria parasites, about one third had some degree of hematuria, and >99% were infected with at least one geohelminth. Hookworms and *Trichoris trichuria* were especially common.

Cross-sectional patterns of stunting and wasting. In the cross-sectional data at base line, the prevalence of stunting rose dramatically with age, from an overall prevalence of 14% in 7-y olds to 83% in 13-y olds (Fig. 1). It is noteworthy that the increase in prevalence was steady both in the prepubertal and pubertal age ranges. In girls, the prevalence of stunting peaked at age 12 and then apparently began to decline, whereas in boys the prevalence of stunting rose steadily up to age 13. This striking trend in low Z-scores by age was not caused by increasing variability of the Z-score distribution by age. The mean Z-score for the study sample steadily decreased from around −1 at 7 y to −2.7 at 13 y, whereas the standard deviation of Z-scores remained between 0.85 and 1.2 at all ages. In contrast to stunting, the prevalence of wasting was low (~10% or lower) and showed no trend by age or sex.

Longitudinal patterns of growth. The cross-sectional patterns described above might have been created by secular trends in malnutrition of early childhood, or by better-off children enrolling at earlier ages. To determine whether ponderal or linear growth retardation was presently occurring in these children, we compared weight and height increments to reference values. The median growth increments for height in boys (Fig. 2) were distinctly below the reference median, lying around the 5th percentile for 8-y olds, and around the 10th percentile for 9- to 13-y olds. The pubertal growth spurt was not apparent in these boys as a group up to age 13 y. The prevalence of height increments below the 5th reference percentile was ~35% up to age 12, but fell to <10% by 13 y. This drop in low values relative to the reference likely reflects the increased variability in the reference population around their pubertal growth spurt, at a time when the Pembian boys have not yet entered their spurt.

In girls, the height increments were around the 5th percentile in 8-y olds, around the 25th percentile in 9- to 12-y olds, and above the 50th percentile in children 12.5–13.5 y. The pubertal growth spurt appears to occur in these girls 1.5–2 y later than in reference girls. The prevalence of height increments below the 5th reference percentile for girls was nearly 40% at age 8, ~20% at ages 9–10, and <5% after age 11 y.

For both girls and boys, the height increments for the two study intervals (roughly April to October and October to April) were very similar. Apparently, these rather wide sea-

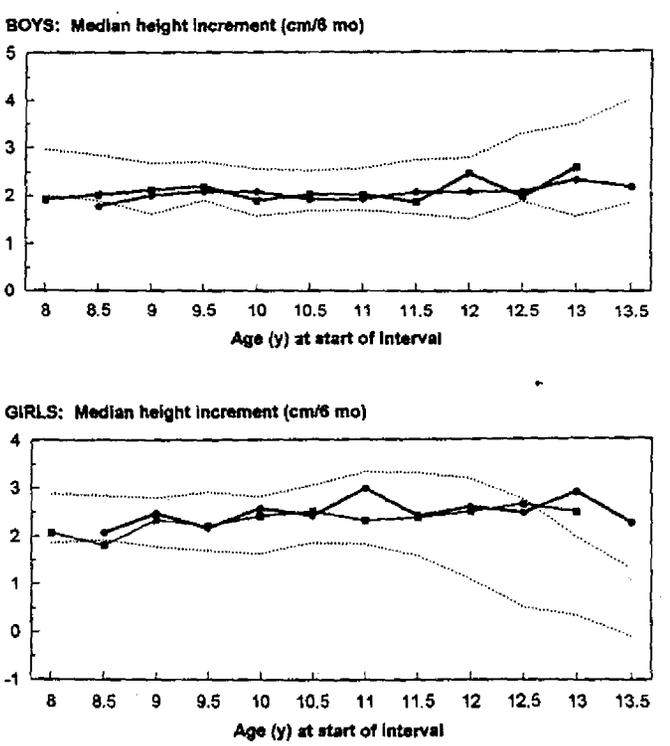

FIGURE 2 Six-month height increments of Zanzibari boys and girls by age. Heavy lines depict the incremental growth curves of Zanzibari children. Squares are median increments from base line to 6 mo in 1072 children, and circles are median increments from 6 to 12 mo in 978 children. Each point represents between 11 and 99 observations. Dotted lines depict the 50th and 5th percentiles from the Fels Longitudinal Study (Baumgartner et al. 1986).

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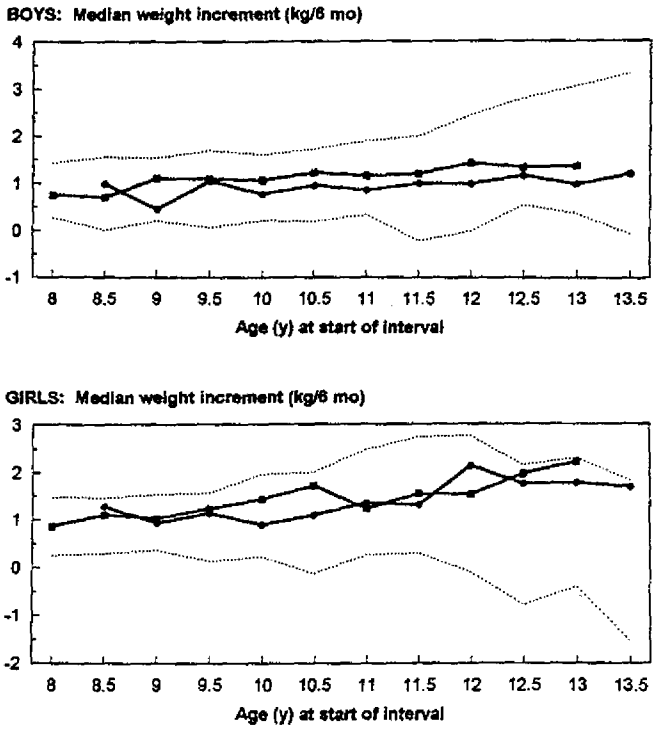

FIGURE 3 Six-month weight increments of Zanzibari boys and girls by age. Heavy lines depict the incremental growth curves of Zanzibari children. Squares are median increments from base line to 6 mo in 1072 children, and circles are median increments from 6 to 12 mo in 978 children. Each point represents between 14 and 106 observations. Dotted lines depict the 50th and 5th percentiles from Fels Longitudinal Study (Baumgartner et al. 1986).

sonal intervals did not capture any seasonal patterns in linear growth.

The weight increments of boys and girls (Fig. 3) were low compared with the reference, lying between the 10th and 25th percentiles and closer to the 25th. There is no evidence of a growth spurt in this group of boys up to age 13.5 y. The weight increments of girls improved with age relative to the reference. Below age 11, girls' weight increments were between the 10th and 25th percentiles, but after age 11 gradually approached the reference median.

The magnitude of growth retardation in the Pembian children can be examined in **Figures 4** and **5.** The base-line and 12-mo follow-up data have been converted into mean differences from the National Center for Health Statistics (NCHS) reference so that the longitudinal and cross-sectional patterns of growth retardation can be compared in real terms. The cross-sectional picture in boys and girls confirms the patterns of prevalence of stunting by age seen in Figure 1.

In boys, the longitudinal picture of growth was similar to the cross-sectional picture, both for weight and for height. Apart from the group of boys 9.25–9.75 y at base line, whose growth tended to be better, boys' weight and height declined steadily away from the reference. Boys lost on average 1.99 cm/y (95% confidence limits: −2.16, −1.82) and 1.97 kg/y (95% confidence limits: −2.11, −1.83) relative to the reference population. In girls, the longitudinal fall away from the reference values was less steep than the cross-sectional trend suggested. Girls began to stop losing height and weight relative to the reference at ages 12–13 y. Still, girls lost on average 1.42 cm/y (95% confidence limits: −1.64, −1.19) and 1.63 kg/y (95% confidence limits: −1.81, −1.45) compared with the reference. Based on the average annual losses from age 8–14 y, the cumulative height deficit was 11.9 cm for boys and 8.5 cm for girls. The cumulative weight deficit in the same period was 11.8 kg for boys and 9.8 kg for girls.

Predictors of poor growth. In addition to the strong relationships between age and sex and growth increments, several base-line morbidities were predictive of smaller growth increments in the succeeding 6-mo period (**Table 2**). Children infected with *A. lumbricoides* had 6-mo weight gains 140 g less than their uninfected peers. Among children < age 11 y, lower hemoglobin concentration was predictive of lower weight gain. This association was strongest using a hemoglobin cut-off of 100 g/L; children below this cut-off had lower weight gains (170 g/6 mo) than those with higher hemoglobin. For both ascariasis and low hemoglobin, the weight increments of normal children were just above the 25th percentile, and those of affected children were just below the 25th percentile.

An association between hematuria and poor linear growth increment was only marginally significant in boys, but it is noteworthy because of its relationship to treatment. This association was found only with microhematuria, which was not treated. Visual hematuria, indicative of severe infection, was treated with a curative dose of praziquantel, and these treated boys exhibited growth similar to their uninfected peers. The linear growth of boys without hematuria was close to the 10th percentile, whereas that of boys with microhematuria was close to the 5th percentile.

DISCUSSION

We found that Pembian school children experienced significant linear growth retardation during their primary school

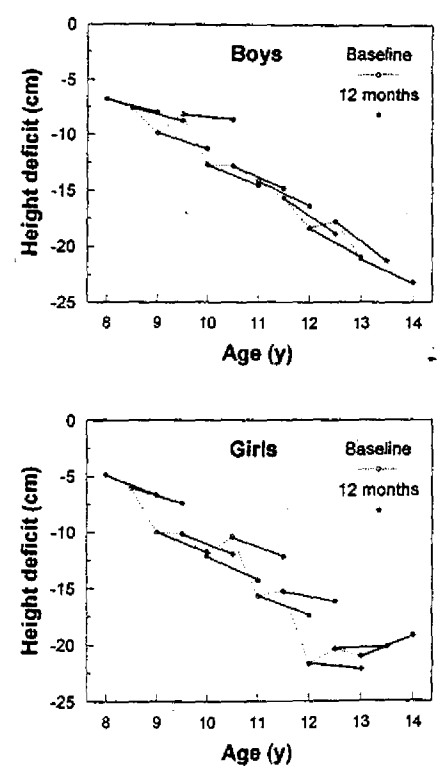

FIGURE 4 Mean difference in height of Zanzibari boys and girls from NCHS/WHO growth reference (World Health Organization 1983). Dotted lines show cross-sectional pattern of height deficit by age (n = 1090), and solid lines show longitudinal pattern (n = 978) for each 6-mo age cohort. Each point represents between 14 and 107 observations.

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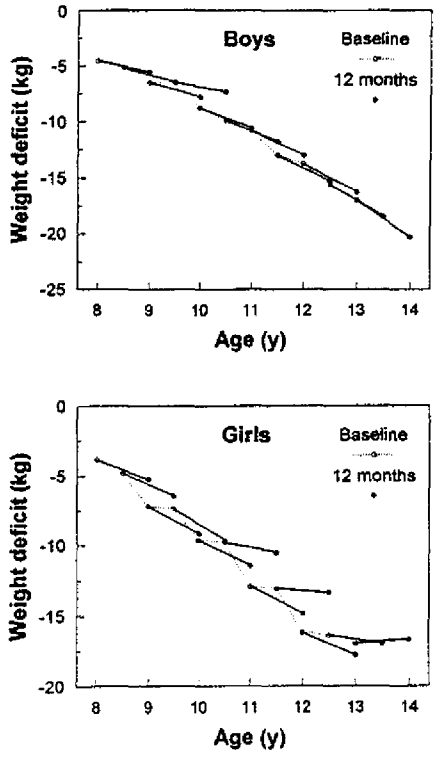

FIGURE 5 Mean difference in weight of Zanzibari boys and girls from NCHS/WHO growth reference (World Health Organization 1983). Dotted lines show cross-sectional pattern of weight deficit by age (n = 1090), and solid lines show longitudinal pattern (n = 978) for each 6-mo age cohort. Each point represents between 14 and 107 observations.

years. This raises several important questions. Could this be a spurious finding? If it is not, is this population unique, or might the same pattern be observed in other African populations? Finally, what is the cause of stunting in this age group and can it be prevented by nutrition or other health interventions during the school years?

Validity of the findings. We have considered several factors that might lead to spurious findings from a survey of African school children. We conclude that our findings are valid, but the issues merit discussion. A single international growth reference is now accepted for assessing growth of children below the age of 5 y (WHO Expert Committee 1995); however, there is little guidance for assessing the growth of children aged 5–10 y. A WHO expert committee recently evaluated the use and interpretation of anthropometry throughout the life span, and children 5–10 y were the only age group not addressed (WHO Expert Committee 1995). Certainly racial differences in growth become significant during late childhood. Evidence suggests that well-nourished children of African Bantu descent (such as the Zanzibaris) grow at least as quickly as white U.S. children during the school years. For example, in the NHANES II survey, 8- to 10-y-old African-American children were on average about 1 cm taller than white children (Eveleth and Tanner 1990). Thus, racial differences are unlikely to explain the difference in growth between the study children and the NCHS or Fels references.

Age is difficult to ascertain in African populations. In the context of the school survey, we could not discern with parents the child's age by use of a local events calendar; rather we relied on the school records. If our age data were very imprecise, the variability of children's Z-scores by age would be inflated. This was not the case. The standard deviation of children's Z-scores fell between 0.85 and 1.20 for all age groups. This is the expected variability based on many anthropometric surveys in developing countries (WHO Expert Committee 1995).

As mentioned previously, schools may provide a biased sample of children. In Zanzibar, school entrance rates are around 80%, but drop out rates are such that overall primary school enrollment is believed to be around 65% (H. M. Chwaya, unpublished data). Although patterns of school attendance have not been described for this particular population, we expect that better-off children enroll at earlier ages and remain in school longer. Because we sampled children only in the first three grades of school, the bias in age at enrollment is probably a greater factor in this sample than bias in drop-out rates. If better-off children enroll at earlier ages, then the youngest children in our sample tend to represent better-off-than-average children, whereas the older children in our sample tend to represent poorer-than-average children, because better-off 12-y olds would have moved beyond the 3rd grade and are no longer observed in our surveys. This bias probably explains why the cross-sectional trend of increased stunting by age is greater than the longitudinal trend of stunting by age. It would also explain why the prevalence of stunting in the 7-y olds is only 14%, far below the 40–50% rates typical of Africa (UN ACC/SCN 1992).

Our 1-y longitudinal follow-up period allowed us to avoid this cross-sectional bias when describing 6- or 12-mo growth increments. However, when we add up the yearly growth defi-

TABLE 2

Health characteristics associated with poor growth in Zanzibari school children

Base-line characteristic	Growth increment[1] (6 mo)	Significance level of regression coefficient
	weight (kg)	
Ascariasis		
Absent	1.18	
Present	1.04	P = 0.046
Age <11 y[2]		
Hemoglobin ≥ 100 g/L	1.18	
Hemoglobin <100 g/L	1.01	P = 0.026
Age ≥11 y		
Hemoglobin ≥100 g/L	1.89	
Hemoglobin <100 g/L	1.95	NS[3]
	height (cm)	
Boys[4]		
No hematuria	2.05	
Microhematuria	1.88	P = 0.073
Visual hematuria[5]	2.11	NS
Girls		
No hematuria	2.57	
Microhematuria	2.55	NS
Visual hematuria[5]	2.44	NS

[1] Increments are from base line to 6 mo, n = 1072. Adjusted for school, age, and sex and, for weight increment only, stunting at base line. Multiple adjusted R^2 was 0.091 for weight model and 0.124 for height model. Values for weight increments are for boys. Values for height increments are for children <11 y.

[2] Significance level of age interaction, P = 0.12.

[3] Not significant, P > 0.10.

[4] Significance level of sex interaction, P = 0.17.

[5] These children were treated with praziquantel.

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cits observed in children of different ages, we are again subject to this bias. In this case, this bias would tend to underestimate the cumulative growth deficits experienced by typical Zanzibari children, because typical Zanzibari 8-y olds would tend to grow more poorly than the subgroup of 8-y olds who were privileged enough to be in our school-based sample. Secular trends in feeding practices and infections during infancy may also contribute to the cross-sectional pattern of stunting by age, but could not create the longitudinal deficits we observed.

Differences in the maturation rate of poorly nourished children compared with reference populations make the interpretation of growth data in this age range problematic. Sexual maturation was not assessed in this study. Modern-day studies of African children have found the median age at menarche to be 13.1–14.5 y, which represents a delay of around 1.5 y compared with U.S. blacks (Eveleth and Tanner 1990). A delay of 1–2 y in the start of the growth spurt of Zanzibari children is also suggested by the pattern of median growth increments we observed in girls. If the difference in growth increments between the study sample and the reference population was caused by the difference in their maturation, then the growth increments would be normalized by shifting the curve of growth increments by age of the Pembian children 1–2 y to the left in Figures 2 and 3. This does not normalize the growth increments. The median height increment for the boys in our sample was close to 2.0 cm from age 8 to 12 y. This does not resemble typical linear growth for reference children of any age from birth to 15 y. The lowest reference median height increment in this age range is 2.52 cm, observed between age 10.5 and 11.0 y (Baumgartner et al. 1986).

Perhaps the greatest evidence for the validity of our findings is their internal coherence. On the basis of the cross-sectional pattern of stunting by age, the incremental growth patterns or the longitudinal differences in height of age cohorts relative to the NCHS reference, the phenomenon of stunting is clear. Although the cross-sectional analysis at base line overestimates the magnitude of stunting, the pattern of stunting by age and sex is consistent.

Comparability to other African studies. Several studies of primary school-age children in Bantu populations are available for comparison with our data. As part of the Nutrition Collaborative Research Support Program (NCRSP), longitudinal growth in a rural Kenyan population (Embu, Kenya) was measured (Neumann and Harrison 1993). In young infants, toddlers and school-age children, linear growth retardation was found. To obtain the school-age data, a community-based sample of 7-y olds was followed prospectively for 18 mo. These children started off near the NCHS 3rd percentile for height-for-age (7 cm shorter than the reference), and ended 18 mo later well below the 3rd percentile (11 cm shorter than the reference). This rate of height loss is steeper than the loss we observed in Pembian 8-y olds, perhaps because our school-based sample selected for better-off 8-y olds. As we found in Zanzibar, Kenyan boys grew more poorly than girls, with height increments around the 3rd and 25th percentile, respectively, for the sexes.

Lawless et al. (1994) conducted an iron supplementation trial in 87 primary school children, ages 6–11 y, from the Kenyan coast (Kwale District), where endemic parasitic infections are very similar to Zanzibar. Although this study was too small to allow an examination of linear growth at different ages, the average height-for-age Z-scores of control children fell from −1.26 to −1.34 in the 14-wk study period, a small but significant decline. The average height gain of control children over the period was 1.1 cm. This would extrapolate to 1.9 cm/6 mo, a value very similar to the increments we observed. No comparison was made between boys and girls.

The growth of children from 2–18 y in rural Machakos District, Kenya, was studied by means of a cross-sectional survey (Stephenson et al. 1983 in Eveleth and Tanner 1990). The sample of preschool children was community-based, but the older children were from two village schools. The mean length of 2-y old children was already 7.3 cm shorter than the NCHS reference. On average, children from the ages of 2–5 y "lost" 1.0 cm/y (total: 3.0 cm), and those from the ages of 6–11 y "lost" 1.5 cm/y (total: 7.5 cm). This pattern of height by age in the school children may be exaggerated because of school enrollment biases, although the authors claimed that below the age of 14 y, the school children were representative of the community. By age 17, the height deficit was 18.2 cm in boys, compared with 12.7 cm in girls. These older children were not representative of the community, but rather tended to come from more privileged families.

Thus it appears that the Pembian children are not unique. These studies provide evidence that African children over the age of 3 y do not grow parallel to reference populations and that boys are more vulnerable to stunting than girls.

Possible etiologies and preventive measures. We hypothesized that stunting in African school-age children may be caused by energy deficiencies, micronutrient deficiencies, or chronic parasitic infection. However, because wasting did not affect a large proportion of children in this population, energy insufficiency is unlikely to be a major cause of stunting.

Iron status is very low in these children, but low hemoglobin was the only base-line indicator of iron deficiency that was associated with growth, and ponderal growth only. However, iron supplementation caused a significant increase in linear growth of children from coastal Kenya, based on the randomized controlled trial mentioned previously (Lawless et al. 1994). Iron-supplemented children gained on average an additional 0.3 cm/14 wk, equivalent to 0.6 cm/6 mo. This amount of additional height gain would bring the rate of height gain close to reference values.

The NCRSP study of Embu, Kenyan children also suggests that dietary etiologies of stunting are operating in this age group. In multivariate models that included maternal height and socioeconomic status, dietary energy intake was significantly associated with attained height at age 8 y, and animal protein intake was significantly associated with height increment (Neumann and Harrison 1993). Animal protein intake might indicate better overall protein intakes, better dietary iron availability or more bioavailable zinc. A higher phytate:zinc molar ratio was also associated with poor linear growth in these children. Inadequate dietary zinc has also been documented in Ghana and Malawi (Ferguson et al. 1993).

Our data suggest that parasitic infections may also contribute to stunting, although the relationships we observed were not statistically very strong, and they explained only a small fraction of the variability in growth. Two trials of anthelminthic treatment with albendazole (effective against the geohelminths: hookworms, *T. trichuria*, and *Ascaris lumbricoides*) (Stephenson et al. 1989 and 1993) and one trial of treatment with metrifonate and praziquantel (effective against the geohelminths and schistosomiasis) (Latham et al. 1990) found positive effects on height gain in primary school children on the Kenyan coast, whereas another publication from one of the same albendazole trials reported no effect on height gain.

A remarkable aspect of helminth infection and malaria is their chronicity. In Zanzibar and many parts of Africa, it is rare to find a child who is not infected with at least one of these parasites throughout their entire childhood and adoles-

cence. Even small decrements in growth may become large when accumulated over 10–12 y.

Slow rates of linear growth in the primary school years might have either of two implications for health. First, the process of stunting might be associated with concurrent risks to the health and development of school-age children, as is the case with young children who experience stunting (WHO Expert Committee 1995). Second, school-age stunting might result in shorter adult height, which decreases work capacity and increases reproductive risks for women (WHO Expert Committee 1995). We cannot describe the effect of the growth retardation we observed on final adult heights, because we did not measure growth during and after puberty. A portion of the height deficit we observed will be compensated by a longer duration of prepubertal growth. Between the ages of 8 and 14 y, Pembian boys are gaining around 4 cm/y, while "losing" 2 cm/y relative to the reference. A delay in puberty of 2 y relative to the reference population would allow them to recover 8 of the 12-cm deficit accrued in this period. A pubertal delay of 3 y (i.e., mean age at menarche of 16–16.5 y) would be needed to compensate fully the deficit we observed. This seems unlikely, based on African values in the literature (Eveleth and Tanner 1990).

It has been suggested from cross-sectional data that rural Kenyan stunted children catch up fully during late adolescence (Kulin et al. 1982). However, this evidence is based on observations of seven 18-y-old boys and eighteen 17-y-old girls compared with reference data collected by other investigators in 1961. Compared with the NCHS reference data, these Kenyan boys had an average height-for-age Z-score of −1.55 at 10 y and −2.74 at 17 y, whereas the girls improved from −2.57 to −1.14.

Our findings raise several new questions: 1) How much of the height deficit accrued during the primary school years is made up through longer prepubertal growth or during the pubertal growth spurt? 2) What are the functional implications of slower linear growth during the primary school years; for example, do these slower-growing children also have slower social or cognitive development? 3) Is school-age stunting preventable through school-based or other public health interventions, and will these interventions correct the functional decrements that might be associated with stunting in this age group? Further research is required to describe the growth of African children based on community-based samples and to evaluate potential interventions to prevent stunting in African school-age children.

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School-Based Deworming Program Yields Small Improvement in Growth of Zanzibari School Children after One Year[1,2,3]

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ABSTRACT Efficacy trials of antihelminthic therapies conducted in Africa have reported improvements in children's growth, but nutritional evaluations of large-scale deworming programs are lacking. We evaluated the first-year effect on growth of a school-based deworming program in Zanzibar, where growth retardation occurs in school children. Children in four primary schools were given thrice-yearly mebendazole (500 mg) and compared with children in four schools that received twice-yearly mebendazole and children in four non-program schools. Evaluation schools were randomly selected and allocated to control, twice-yearly or thrice-yearly deworming. Approximately 1000 children in each program group completed the 1-y follow-up. Children <10 y old gained 0.27 kg more weight ($P < 0.05$) and 0.13 cm more height ($P = 0.20$) in the twice-yearly group, and 0.20 kg more weight ($P = 0.07$) and 0.30 cm more height ($P < 0.01$) in the thrice-yearly group, compared with the control group. Children <10 y old with higher heights-for-age at baseline had higher weight and height gains in response to deworming. In children ≥10 y old, overall program effects on height or weight gains were not significant. But in this age range, younger boys had significant improvements in height gain with thrice-yearly deworming, and children with higher heights-for-age had greater improvements in weight gain with deworming. We conclude that the deworming program improved the growth of school children, especially children who were younger and less stunted, but the improvements were small. More effective antihelminthic regimens or additional dietary or disease control interventions may be needed to substantially improve the growth of school children in areas such as Zanzibar. J. Nutr. 127: 2187–2193, 1997.

KEY WORDS: • *humans* • *Africa* • *growth* • *geohelminths* • *hookworms*

School-based deworming has been advocated as a highly cost-effective public health measure in less-developed countries (World Bank 1993). It is estimated that over 1 billion people are infected with *Ascaris lumbricoides*, 880 million with hookworms, and 770 million with *Trichuris trichiura* (Warren et al. 1993), and school children are the group most heavily infected with these geohelminths (Savioli et al. 1992). The expected benefits from deworming in this age group are improved school participation and performance, growth and iron status (Warren et al. 1993).

Improved growth of primary school children could result in greater adult height, which is associated with greater work capacity in men and women and with improved reproductive outcomes in women (WHO Expert Committee 1995). We previously published evidence that Zanzibari school children experience significant linear growth retardation compared with international growth references (Stoltzfus et al. 1997a). Because helminth infections are highly prevalent in Zanzibar, the growth of school children might be improved if helminth infections were controlled.

Several efficacy trials of antihelminthic therapies in school children have produced promising results (Kruger et al. 1996, Latham et al. 1990, Stephenson et al. 1989, 1993a and 1993b), especially in coastal East Africa, where helminth infections are prevalent and intense. In these controlled randomized trials, growth, appetite, fitness and iron status improved in children treated with antihelminthic drugs. However, evidence is lacking in the literature on the program effectiveness of large-scale school-based deworming efforts.

We report here the results of an evaluation of a school-based deworming program implemented by the Ministries of Health and Education of Zanzibar on Pemba Island. The evaluation measured the effect of the program on helminth infections, nutritional status and school attendance and compared the effects of two deworming schedules, twice-yearly and

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thrice-yearly. A single 500-mg dose of generic mebendazole was chosen as the antihelminthic treatment because of its simplicity and low cost (US$0.027 per dose, Albonico et al. 1994). We describe the effect of twice-yearly and thrice-yearly deworming on the height and weight gains of primary school children in Zanzibar.

METHODS

The school-based deworming program. The Zanzibar school-based deworming program began in 1994 on Pemba Island, the smaller of the two islands of Zanzibar, a part of the United Republic of Tanzania. From 1988 to 1992, a school-based "test-and-treat" program was conducted on Pemba Island for the control of urinary schistosomiasis (Renganathan et al. 1995). This program had been implemented by the Pemba Island Helminth Control Team, a unit of the Ministry of Health, in cooperation with the Ministry of Education and local school teachers. Surveys conducted by the Helminth Control Team showed that geohelminth infections were also highly prevalent throughout Pemba (Renganathan et al. 1995). Building on the success of the test-and-treat campaign for schistosomiasis, a school-based deworming program to control intestinal parasites was planned.

On the basis of a trial of antihelminthic drugs conducted in Pemba (Albonico et al. 1994), a single 500-mg dose of generic mebendazole was chosen as the regimen to be used in the program. Although mebendazole did not control hookworm infection as well as albendazole, its effect on *A. lumbricoides* and *T. trichiura* infections was similar to that of albendazole, and the cost of mebendazole was around 10% the cost of albendazole at that time. A thrice-yearly regimen was chosen because of the rapidity of reinfection with helminth infections (Albonico et al. 1995) and the limited efficacy of mebendazole against *T. trichiura* and the hookworms.

Design of the program evaluation. Because the deworming program was planned to be extended to Zanzibar Island and sustained for the indefinite future, a controlled evaluation of its effect was justified, even though this meant delaying the start of the program in a small number of schools. Also, a twice-yearly deworming regimen was evaluated along with the thrice-yearly regimen of choice, to provide evidence to the Ministry of Health as to whether a twice-yearly regimen would be adequate to improve nutrition outcomes and school attendance. The evaluation was planned before the start of the deworming program in any school so that an optimal research design could be implemented.

Of the 72 public primary schools on Pemba Island, 12 schools were randomly selected for evaluation (**Fig. 1**). Pemba is divided into four districts of roughly equal size, and the selection of schools was stratified by district, so that three schools in each district were chosen. Within districts, the schools were randomly allocated to the control, twice-yearly or thrice-yearly group. All children within a school received the same program regimen. For logistical reasons, only morning classes were selected for the survey. Although the deworming program was administered to all classes of grades 1–5, only classes of grades 1–4 were included in the evaluation of nutritional effect, because malnutrition was expected to be of greatest importance in younger children. Grades 1 and 2 were deliberately overrepresented for the same reason.

Baseline, 6-mo and 12-mo follow-up nutrition and parasitology surveys were conducted in March–May 1994, October–November 1994 and March–May 1995, respectively. The design was based on the comparison of within-individual changes in childrens' nutritional status from baseline to 12 mo in each treated group vs. the placebo group, and these are the analyses presented in this article. The 6-mo follow-up survey was planned to provide an interim measure of program effect for evaluation of whether a nontreated group could be maintained ethically. The full battery of assessments was not completed at 6 mo, and those data are not presented here. A sample size of 1000 children per group was estimated to be sufficient to compare the twice-yearly and thrice-yearly program groups with the control group, accounting for the design effect of randomizing at the school level and planning for subgroup analyses.

Before the study began, meetings were held at each school to inform parents of the deworming regimen that would be implemented in their school, the purpose of the evaluation, its risks and benefits, and alternatives to participation in the surveys. All children present in school on survey days were invited to participate in each survey. Children found to have hemoglobin <70 g/L (3.5% of children) were treated with mebendazole and oral iron supplements. Inclusion or exclusion of these children did not affect the growth results, and they are included in the analyses presented here. Overall, 91% of children listed on teachers' class registers participated in the baseline survey, and 85% of those children were assessed again at the 12-mo follow-up, in the following school year (Fig. 1). Compared with children who completed the study, those lost to follow-up were similar in all measured characteristics except that they were more likely to be boys (60.0% vs. 50.3%, $P < 0.001$) and less likely to be stunted (height-for-age Z-score < -2, 42.1 vs. 48.6%, $P < 0.005$). Both of these characteristics were associated with greater growth effect from deworming (see Results); thus, any bias caused by loss to follow-up would act to diminish the effect that we measured. The study was approved by the institutional review boards of The Johns Hopkins University, the World Health Organization and the Ministry of Health of Zanzibar.

Nutritional and parasitologic assessments. Nutritional assessment methods have been described in greater detail elsewhere (Stoltzfus et al. 1997a and 1997b) but are briefly reported here. Children's weights were measured to the nearest 0.1 kg using a battery-powered digital scale (Seca Inc., Columbia, MD), and heights were measured to the nearest 0.1 cm using a wooden stadiometer (Shorr Productions Inc., Olney, MD). Children were lightly clothed and shoes were removed. Age was calculated from the birth date on school records. When this was missing, the child's self-reported age was used. A venous blood sample was drawn to assess micronutrient status. Hemoglobin was determined using the HemoCue method (HemoCue AB, Angelhom, Sweden), and erythrocyte protoporphyrin was determined using an fluorometer (Aviv Biomedical, Lakewood, NJ). Serum ferritin was determined using an immunoassay (DELFIA System by Wallac Inc., Gaithersburg, MD).

Parasitological methods are described in greater detail elsewhere (Stoltzfus et al. 1997b), and are briefly recounted here. Stool containers were given to children on the day before the survey, and they were instructed to bring a sample of their stool to school the next day. About 95% of children returned stool samples in each survey. Quantitative counts of helminth eggs in feces were determined by the Kato-Katz method (WHO 1994). Urine samples were collected from 100% of children. Microhematuria was determined using Hemastix test strips (Ames Laboratories, Elkhart, IN) and was used as a proxy indicator of urinary schistosomiasis (Savioli et al. 1990). Thick and thin blood films were stained with giemsa, and malaria parasites were counted against leukocytes using standard methods (WHO 1991).

Data analysis. The study was designed to detect differences in the twice-yearly or thrice-yearly treated group compared with the placebo group; however, our sample size was not designed to detect differences in effect between twice-yearly and thrice-yearly deworming. Thus we present statistical tests of these two comparisons, rather than comparisons among the three groups.

First, baseline characteristics of the treatment groups and the placebo group were compared using simple linear regression. Characteristics that differed to a potentially biologically important degree were controlled for in subsequent tests of program effect.

The measures of program effect on growth were the 12-mo weight and height increments for each child. All analyses were performed at the individual level and were adjusted for within-school correlations using the generalized estimating equations approach (Diggle et al.). Some baseline characteristics of children in the three program groups differed at baseline (Table 1). Thus, the overall 1-y program effects on weight and height increments of children in twice-yearly and thrice-yearly dewormed schools were compared with those in control schools, using multiple linear regression to adjust for characteristics that differed at baseline.

Given the heterogeneity of school children, we expected that some subgroups of children would benefit more from deworming than others. Multiple linear regression models with program group interaction terms were used to test whether program effects were greater in children with certain baseline characteristics. Baseline characteristics

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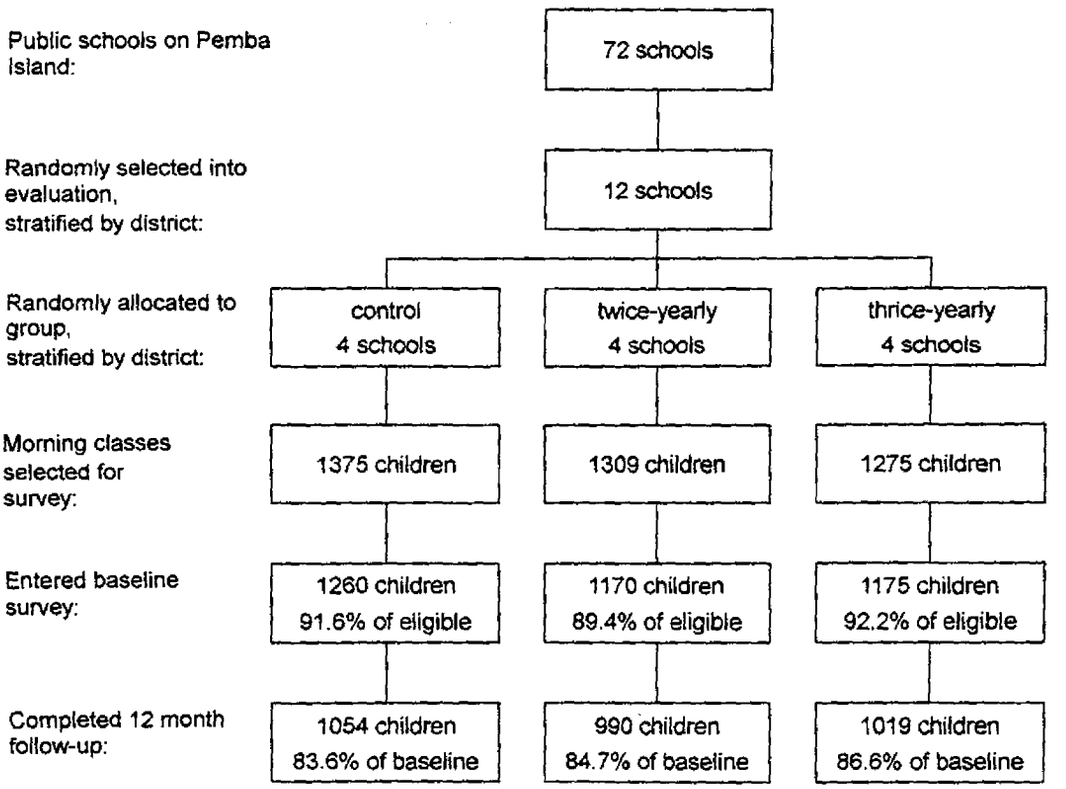

FIGURE 1 Schema of research design, including numbers of children in each program group at each step of the evaluation.

associated with greater benefit from the program are termed predictors of benefit. Interactions with program group were considered potentially important if they were significant at P levels <0.15 and if they were consistent in both program groups.

Analyses were conducted separately for children <10 y and ≥10 y of age, to separate the effects on pre-pubertal and pubertal growth. Sexual maturation was not assessed. Height-for-age Z-scores were determined using the anthropometric subroutines of EpiInfo, version 6 (Centers for Disease Control and Prevention, Atlanta, GA). Weight-for-height Z-scores and body mass index [BMI, weight (kg)/height^2 (m^2)] percentiles were considered as indicators of thinness. Body mass index is presented here because it is recommended for assessing children >10 y of age, whereas weight-for-height Z-score is not (WHO Expert Committee 1995), and there are no recommendations for assessing thinness in children 6–10 y old. The BMI percentiles were defined by the sex and race-specific tables of Must et al. (1991a and 1991b). Analyses were performed using Systat (SPSS, Chicago, IL) and SAS (SAS Institute, Carey, NC) statistical software.

RESULTS

Baseline characteristics of the study sample. The study sample was composed about equally of boys and girls, whose average age was 10 y (Table 1). Helminth infections were nearly universal. Around 95% of children were infected with hookworms and *T. trichiura*, and about two-thirds were infected with *A. lumbricoides*. Around 60% had circulating malaria parasites and about one-third had microhematuria, an indication of urinary schistosomiasis. Stunting and low BMI were common and increased with age. The iron status of the population was very poor. The prevalence of anemia (hemoglobin <110 g/L) was 62.3%, and 82.7% of anemia was associated with iron deficiency (protoporphyrin >90 μmol/mol heme or serum ferritin <18 μg/L) (Stoltzfus et al. 1997b).

There were several significant differences among the three program groups at baseline (Table 1). This is not surprising, because only 12 schools were randomly allocated. Both program groups had more hookworm and *T. trichiura* infection than the control group, but the twice-yearly group had less *A. lumbricoides* infection than the other groups. In terms of anthropometry, the twice-yearly group was less stunted than the other two groups at baseline.

Program effect on helminth infections. There was a clear dose response with frequency of deworming on all three helminths (**Table 2**). Ascariasis was effectively controlled, but hookworms and *T. trichiura* were controlled less well. These deworming regimens reduced the intensity of *T. trichiura* and hookworm infections but had little effect on the prevalence of infection.

Overall program effect on growth. Adjusted for baseline characteristics, the 12-mo weight gains were ~2 kg for children <10 y old and 3 kg for children ≥10 y old (**Table 3**). In children <10 y old, the twice-yearly group had weight gains 0.27 kg greater than those of the control group ($P < 0.05$). The thrice-yearly group had weight gains 0.20 kg greater than those of the control group, but this difference was not significant ($P = 0.07$). There were no significant program effects on weight gains in children ≥10 y of age. In children <10 y old, the 12-mo height gains in the thrice-yearly group were 0.30 cm greater than in the control group ($P < 0.01$). In children ≥10 y of age, there was no significant program effect on height.

Predictors of benefit. As expected, children with certain characteristics had a greater growth response to deworming. Among children <10 y old, the degree of stunting was the only predictor of benefit (**Fig. 2**). Children who had higher height-for-age Z-scores at baseline benefited more from deworming. On the basis of the multiple regression model with

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TABLE 1

Baseline characteristics of study children by program group[1]

Characteristic	Program group		
	Control	Twice-yearly deworming	Thrice-yearly deworming
	(n = 1054)	(n = 990)	(n = 1019)
Male sex, %	51.2	50.4	49.4
Age, *y*	10.5 ± 1.8	10.7 ± 1.8*	10.5 ± 1.8
Hookworms			
Infected, %	91.2	94.5*	95.8*
Eggs/g feces	332 (48, 2304)	505 (73, 3458)*	601 (86, 4204)*
T. trichiuria			
Infected, %	94.7	96.7*	97.3*
Eggs/g feces	531 (95, 2949)	577 (104, 3186)	625 (115, 3398)
A. lumbricoides			
Infected, %	73.0	66.5*	76.4
Eggs/g feces	239 (7, 8642)	149 (4, 5307)*	335 (9, 11,959)
Malaria parasitemia, %	57.3	57.0	65.6*
S. haematobium, %	31.9	21.3*	39.5*
Age <10 y	(n = 473)	(n = 442)	(n = 476)
HAZ	−1.44 ± 1.30	−1.21 ± 1.30*	−1.44 ± 1.31
HAZ < −2, %	30.7	26.0	36.1
BMI	14.4 ± 1.2	14.3 ± 1.2	14.4 ± 1.2
BMI < 5th centile, %	22.6	22.1	21.9
Age ≥10 y	(n = 581)	(n = 548)	(n = 543)
HAZ	−2.33 ± 1.25	−2.24 ± 1.24	−2.44 ± 1.24
HAZ, < −2, %	64.0	59.6	66.3
BMI	14.8 ± 1.4	15.1 ± 1.4*	15.0 ± 1.4
BMI < 5th centile, %	48.9	48.4	43.5

[1] Values are means ± SD. For measures of eggs per gram of feces, values are geometric means (−1 SD, +1 SD); zero values were included, with log-transformed values defined as zero. * Significantly different from corresponding value for the control group ($P < 0.05$). HAZ = height-for-age *Z*-score, BMI = body mass index (kg/m^2).

height-for-age Z-score as a continuous variable, children with height-for-age Z = 0 gained 0.44 kg and 0.39 cm more in the twice-yearly group and 0.46 kg and 0.51 cm more in the thrice-yearly group than children in the control group (all comparisons to control group, $P < 0.005$). In children with height-for-age Z = −2, the only benefit was 0.23 cm greater height gain in the thrice-yearly group compared with the control group ($P < 0.05$).

Among children ≥10 y old, shortness-for-age again predicted weight gain from deworming (**Fig. 3**). Children who were less stunted (higher height-for-age Z-score) at baseline gained more weight in response to deworming. Also, in this age group, age and sex predicted height gain from deworming. Girls grew less than boys in response to deworming, and the growth response was greatest in younger boys. Ten-year-old boys gained 0.31 cm more in the twice-yearly group ($P < 0.020$) and 0.49 cm more in the thrice-yearly group ($P < 0.025$) compared with the control group.

The presence or intensity of the three geohelminth infections at baseline did not predict benefit from the deworming program for either weight or height gain, nor did iron deficiency or anemia.

TABLE 2

Prevalence and intensity of helminth infections by program group at 12-mo follow-up survey

	Program group[1]		
	Control	Twice-yearly deworming	Thrice-yearly deworming
A. lumbricoides			
Prevalence, %	84	58	38
Eggs/g feces	653 (25, 17,367)	54 (2, 1433)	10 (0, 289)
T. trichiura			
Prevalence, %	97	92	85
Eggs/g feces	788 (96, 6449)	340 (41, 2633)	147 (19, 1256)
Hookworms			
Prevalence, %	96	89	88
Eggs/g feces	778 (94, 6728)	329 (41, 2826)	262 (30, 2044)

[1] Samples sizes per group given in Figure 1. All values for twice-yearly and thrice-yearly groups differ significantly from corresponding values for the control group ($P < 0.05$). For measures of eggs per gram of feces, values are geometric means (+1 SD, −1 SD); zero values were included, with log-transformed values defined as zero.

DISCUSSION

This evaluation provides strong evidence that the school-based deworming program improved the growth of school children. The pre-post design of the evaluation, the comparison between randomly allocated program and control schools, and statistical adjustment for the differences in baseline characteristics among groups enable us to conclude that periodic antihelminthic treatment caused greater height and weight gains among children participating in the deworming program.

The fact that control of helminth infections improved the linear growth of children <10 y old also provides support for our previous inference that Zanzibari children experience lin-

TABLE 3

Overall adjusted 12-mo growth increments in children in control, twice-yearly or thrice-yearly deworming program schools

	Program group[1]		
	Control	Twice-yearly deworming	Thrice-yearly deworming
Weight gain, *kg*			
Age <10 y[2]	2.11 ± 0.08	2.38 ± 0.08*	2.31 ± 0.08
Age ≥10 y[3]	3.01 ± 0.11	3.20 ± 0.12	3.04 ± 0.12
Height gain, *cm*			
Age <10 y[4]	4.29 ± 0.07	4.42 ± 0.07	4.59 ± 0.07**
Age ≥10 y[3]	4.74 ± 0.08	4.67 ± 0.08	4.81 ± 0.08

[1] Samples sizes per group are given in Figure 1. Values are means ± SEM. * Significantly different from corresponding value for the control group ($P < 0.05$). ** Significantly different from corresponding value for the control group ($P < 0.01$).

[2] Adjusted for district, sex, age, and height-for-age *Z*-score.

[3] Adjusted for district, sex, age, height-for-age *Z*-score, and low body mass index.

[4] Adjusted for district, sex, height-for-age *Z*-score and low body mass index.

ear growth retardation during their early school years (Stoltzfus et al. 1997a). If these children were achieving their genetic potential for linear growth in the absence of intervention, then antihelminthic treatment would not have increased their rate of linear growth.

However, the magnitude of the growth response to deworming was small. This can be illustrated in several ways. In children <10 y of age, the growth responses in the thrice-yearly program group were 0.2 kg/y and 0.3 cm/y. Compared with the control group gains, these represent improvements of 10% in weight and 7% in height. The largest height responses to deworming, seen in less stunted children receiving thrice-yearly deworming, were around 0.5 cm/y, or 12% of control height gains. These gains do not bring the growth of Zanzibari school children in line with the international growth reference. An average 9-y-old child tracking at the median of the WHO reference for height would gain 3.7 kg/y and 5.7 cm/y (average of values for boys and girls, from WHO 1983).

Because Zanzibari children will benefit from the school-based deworming program throughout their primary school years, the effect on growth has a long period of time to accumulate. If children gained an extra 0.3 cm/y from the thrice-yearly deworming program, over a 5-y period they might accumulate a height benefit of 1.5 cm. For an 11-y-old boy, this represents an improvement in height-for-age of 0.22 Z-scores (WHO 1983). Even the largest height benefit we observed in a subgroup, 0.5 cm/y, would mean an improvement of only 0.37 Z-scores if sustained over a 5-y period.

A series of smaller randomized placebo-controlled efficacy trials conducted in Kenya, where the epidemiology of geohelminths is very similar to that in Zanzibar, found larger growth responses to antihelminthic treatment than we did. A single 400-mg dose of albendazole improved height gains by 0.6 cm and weight gains by 1.3 kg in a 6-mo trial in 150 first and second graders (Stephenson et al. 1989). Treatment with metrifonate (which treats both hookworms and *S. haematobium*) or praziquantel (which treats only *S. haematobium*) improved height gains by 0.2 cm in a 5-wk trial in 48 pre-pubescent boys infected with both parasites (Latham et al. 1990). A single 600-mg dose of albendazole improved height gains by 0.6 cm and weight gains by 1.0 kg in 53 boys after 4 mo (Stephenson et al. 1993b). However, those boys were part of a larger trial comparing one dose of albendazole in a 8-mo period to two 4-mo doses in 284 boys and girls (Stephenson et al. 1993a). In the larger and longer trial, weight gains improved by 1.0 kg, but there was no effect on height gains.

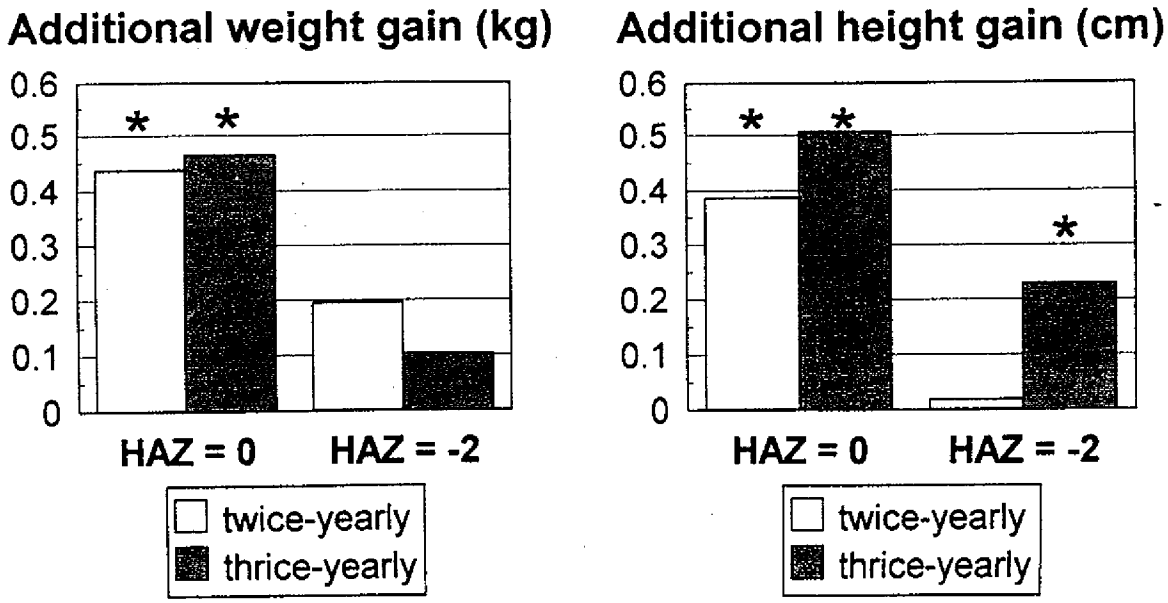

FIGURE 2 Effects of twice-yearly or thrice-yearly deworming on 12-mo weight and height gains of children <10 y of age, according to height-for-age *Z*-score (HAZ) at baseline. The *y*-axis represents the average additional height or weight gain associated with being in the twice-yearly or thrice-yearly dewormed groups compared with the control group, adjusted for sex, age and district. Asterisks indicate weight or height gains that are significantly different ($P < 0.05$) from control group gains at the indicated HAZ value. Interaction between HAZ and program group on weight was significant at $P = 0.047$ for twice-yearly deworming and at $P = 0.005$ for thrice-yearly deworming. Interaction between HAZ and program group on height was significant at $P = 0.008$ for twice-yearly deworming and at $P = 0.041$ for thrice-yearly deworming. Sample sizes per group are given in Figure 1.

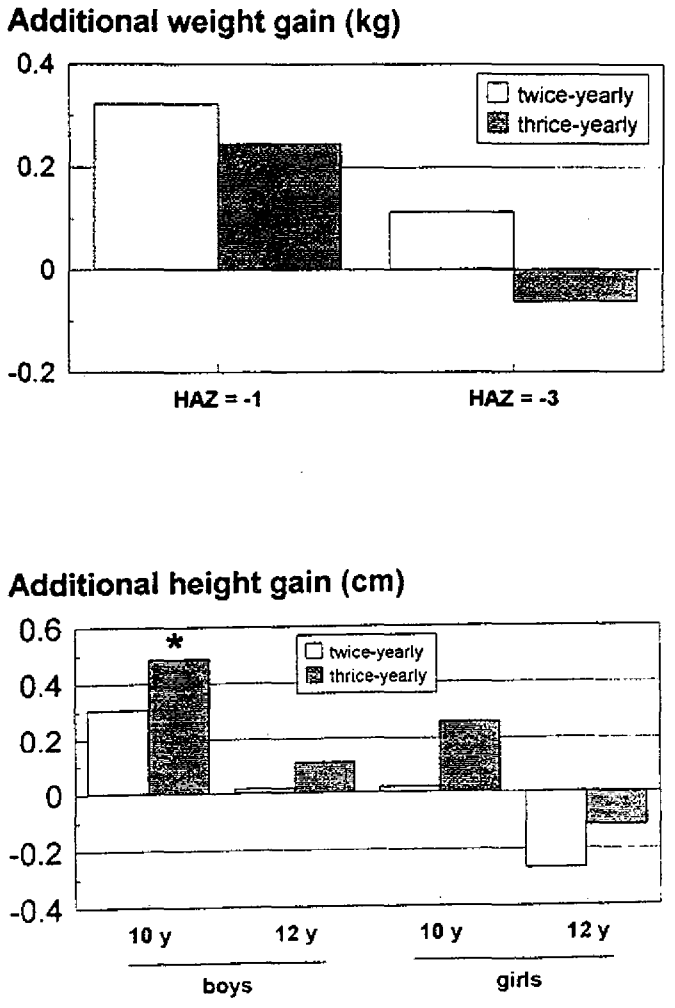

FIGURE 3 Effect of twice-yearly or thrice-yearly deworming on 12-mo weight and height gains of children ≥10 y of age, by baseline characteristics. The *y*-axis represents the average additional height or weight gain associated with being in the twice-yearly or thrice-yearly dewormed groups compared with the control group, adjusted for sex, age and district. Asterisks indicate weight or height gains that are significantly different ($P < 0.05$) from control group gains at the values indicated on the *x*-axis. HAZ = height-for-age *Z*-score. Interaction between HAZ and program group on weight was significant at $P = 0.02$ for twice-yearly deworming and at $P = 0.12$ for thrice-yearly deworming. Interaction between age and program group on height was significant at $P = 0.0001$ for twice-yearly deworming and at $P = 0.002$ for thrice-yearly deworming. Interaction between sex and program group on height was significant at $P = 0.11$ for twice-yearly deworming and was not significant ($P = 0.30$) for thrice-yearly deworming. Samples sizes per group are given in Figure 1.

There are several possible reasons why the growth effect we observed was small. First, there might be other factors limiting the growth of these children in addition to helminth infections, i.e., helminth control is necessary but not sufficient to improve substantially the growth of Zanzibari school children. This explanation is supported by the consistent finding that less stunted children had the greatest growth response to deworming. This effect modification was apparent in both program groups for height and weight gains of children <10 y of age and for weight gains of children ≥10 y of age. If helminth infection were the only factor holding back the linear growth of children, then we would expect to see the most stunted children having the greatest growth response to antihelminthic treatment. We found the opposite to be true, which is consistent with the idea that something in addition to geohelminth infections is slowing the growth of stunted children.

Under conditions found in areas such as Zanzibar, additional zinc or iron may be needed for children to achieve normal growth. A potential role of vitamin A deficiency can be ruled out, because the serum retinol distribution of first-graders in this sample was nearly identical to that of children in the United States (data not shown). These children were significantly iron deficient (Stoltzfus et al. 1997b), and iron supplementation improved the growth of Kenyan school children (Lawless et al. 1994). We have not evaluated zinc status in this population, but zinc deficiency has been reported in other parts of sub-Saharan Africa (Ferguson et al. 1993). In addition to geohelminths, chronic malarial infection or strongyloidiasis might limit the growth of school children. An effective bednet program improved the growth of preschool children in an area of mainland Tanzania with malaria epidemiology similar to that in Zanzibar (Shiff et al. 1996). The prevalence of *Strogyloides stercoralis* infection in a subsample of this study cohort at baseline was 41.2% (Albonico, M., and Cancrini, G., unpublished data), but the nutritional consequences of this infection are largely unknown (Stephenson 1987).

A second possible reason for the small growth effect is that anthelminthic treatment might cause a temporary growth spurt that is not sustained. These school children may have multiple "gates", growth inhibitors or missing growth factors, that limit their rate of growth. Geohelminths may be one such growth inhibitor, but when this burden is alleviated the child can grow at a normal rate only until bumping into the next gate. Thus, when evaluated over a 1-y period, the growth effect we observed seems relatively small compared with results from shorter trials.

This explanation is supported by two trials in the literature. First is that of Stephenson et al. (1993a), in which results after 4 mo showed significant height and weight gains; however, when the 8-mo trial was completed, the weight gain had not increased further and the height gain had disappeared (Stephenson et al. 1993b). However, it should be noted that the 4-mo results were reported on a subgroup of boys who may have responded differently from the total group of boys and girls combined. A recent trial conducted north of Cape Town, South Africa (Kruger et al. 1996) also suggests that the effect of deworming on growth velocity abates over time. Iron-deficient 6- to 8-y-old children who received two 200-mg doses of albendazole 4 mo apart had height gains about 0.6 cm greater after 5 mo than children receiving placebo. After 11 mo, the relative height gain remained around 0.6 cm. This study must be interpreted with caution in this context, because the children were also given iron-fortified soup in a factorial design with deworming, and only 73 children composed the iron-deficient subsample.

To explore this possibility in our own data, we compared the observed growth effect using the 6-mo follow-up data to the 12-mo effects. In children <10 y of age, the weight effects (difference between weight increments in the treated vs. control group) after 6 mo were 0.32 kg ($P < 0.01$) in the twice-yearly group and 0.18 kg ($P = 0.12$) in the thrice-yearly group. These values are similar to the effects observed after 12 mo (see Table 2). The 6-mo height effects from deworming were −0.15 cm ($P = 0.23$) in the twice-yearly group and 0.19 cm ($P = 0.48$) in the thrice-yearly group, values that rather support the assumption that the height effect observed in the thrice-yearly deworming group was gradual throughout the year. These comparisons provide some supporting evidence that deworming causes an immediate increase in weight gain that is not sustained beyond 6 mo. The same does not seem to be true for height. In children ≥10 y of age, the 6-mo

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weight and height effects were trivial, and none was statistically significant.

A third possible explanation is that mebendazole controlled hookworms and *T. trichiura* too poorly to induce a large growth response. Neither of the benzimidazole antihelminthic drugs is very effective against *T. trichiura* (Albonico et al. 1994). However, the trials conducted by Latham and Stephenson and colleagues in Kenya (Latham et al. 1990, Stephenson et al. 1989, 1993a and 1993b) used antihelminthic treatments that are more effective against hookworms, and larger reductions in the prevalence and intensity of hookworm infection were attained. This explanation is supported by the dose response in the effect of twice-yearly and thrice-yearly deworming on height gains. The height gains of children <10 y of age increased with frequency of deworming, both in terms of the overall group effects (Table 2) and the subgroup analyses (Fig. 2 and 3). However, a similar dose response was not seen in weight gains.

If poor control of helminth burdens is the reason for the small growth effects, then hookworms or *T. trichiura* rather than *A. lumbricoides* must be mainly responsible, given that ascariasis was controlled quite effectively by mebendazole. The lack of effect of *A. lumbricoides* on growth is also suggested by the finding that the growth response to antihelminthic treatment did not differ between children with and without *A. lumbricoides* infection at baseline (i.e., ascariasis was not a predictor of benefit from deworming). Over 30% of children did not have ascariasis at baseline; thus we had reasonable statistical power to observe this effect if it existed. This is in contrast to the hookworms and *T. trichiura*, which infected the vast majority of children at baseline.

Finally, the small effect we observed might be due to chance in the particular sample that we used. It is possible that the growth response in this sample of children was at the lower end of effects that will usually result from school-based deworming programs or that the large growth response in the Kenyan trials represents the upper expectation and will probably not be repeated. Additional evaluations of the growth effect of deworming programs are needed to provide reliable expectations of effect for program planners.

In summary, controlling *A. lumbricoides* infection and reducing the intensity of *T. trichiura* and hookworm infections in Zanzibari school children brought about small improvements in weight and height gains, particularly in children <10 y of age and in children who were less stunted. The public health relevance of the growth effect we observed is questionable. It is possible that helminth control is necessary but not sufficient to substantially improve the growth of school children in areas such as Zanzibar, and additional interventions or combinations of interventions should be tested. Antihelminthic regimens that are more effective against hookworms should be evaluated in programmatic settings and over time frames of at least 1 y. Improved growth is only one potential benefit from school-based deworming programs, and a final appraisal of the worth of such programs must also consider school participation, educational performance and effect on iron status.

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T Cell Subsets and Cytomegalovirus Retinitis in Human Immunodeficiency Virus–Infected Patients

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A case-control study was done to investigate the relationship between T cell subsets and cytomegalovirus (CMV) retinitis in human immunodeficiency virus (HIV)–infected subjects with or without CMV retinitis and $CD4^+$ cell counts of $<0.050 \times 10^9$/L. Cell surface markers on peripheral blood lymphocytes were evaluated using flow cytometry. Patients with CMV retinitis had significantly lower levels of $CD8^+$ cells (median: 0.152×10^9/L) compared with levels for controls (median: 0.296×10^9/L, $P < .001$). Significant down-regulation of costimulatory molecule $CD28^+$ and lymphocyte function–associated antigen-1 (LFA-1) expression was observed in patients versus controls ($CD28^+$: 0.048×10^9/L vs. 0.143×10^9/L, $P < .001$; LFA-1: 0.238×10^9/L vs. 0.400×10^9/L, $P < .001$), but no significant differences were noted for NK cells. We propose that progressive loss of the $CD3^+CD8^+$ cell subset and down-regulation of CD28 and LFA-1 accessory molecules are associated with an increased risk of CMV retinitis in HIV-infected patients.

Cytomegalovirus (CMV) disease is assuming a more significant role as people with AIDS live longer. The cumulative lifetime risk of CMV disease is 45% [1], with retinitis being its most common manifestation. CMV retinitis is the most common intraocular infection in AIDS patients and is a cause of significant morbidity. It generally occurs when $CD4^+$ cell counts are $<0.050 \times 10^9$/L [2].

Despite the presence of antibodies to CMV, 30%–40% of AIDS patients develop CMV disease, suggesting that cell-mediated immunity is necessary for maintaining CMV latency. This supposition is also supported in clinical and animal studies in which recovery from CMV infection is dependent on an intact cellular immunity, in particular $CD8^+$ T lymphocytes and NK cells [3].

In addition to the progressive loss of $CD4^+$ and $CD8^+$ T lymphocytes in human immunodeficiency virus (HIV) infection, it was recently reported that CD28 antigen expression declines as HIV infection advances [4]. Because loss of this molecule might partly explain cytotoxic T lymphocyte anergy [5], we postulate that progressive cytotoxic/suppressor T cell (CD8 cell) loss or anergy (or both) could predispose HIV-infected persons to CMV disease. Furthermore, since it is desirable to be able to identify patients at higher risk of CMV retinitis (for primary prophylaxis), we conducted a case-control cross-sectional study on the expression of lymphocyte subset markers in peripheral blood lymphocytes from HIV-infected persons with and without CMV retinitis. We hypothesized that decreased levels of $CD8^+$ cells, NK cells, and costimulatory molecules (CD28 and lymphocyte function–associated antigen-1 [LFA-1]) would predispose individuals with HIV to CMV retinitis.

Materials and Methods

From September 1994 through May 1995, HIV-infected patients with CMV retinitis were recruited from the AIDS Ophthalmology Clinic at Johns Hopkins Hospital. A diagnosis of CMV retinitis was made on the basis of typical ophthalmologic features. HIV-infected patients without CMV retinitis and with $CD4^+$ cell counts $<0.050 \times 10^9$/L were recruited as controls. Patients with visceral CMV disease were excluded from the control group.

Blood was collected for flow cytometry and a complete blood cell count. Blood for flow cytometry was transported at room temperature and prepared within 4 h of collection. Clotted specimens were discarded. Peripheral blood lymphocytes were isolated, stained, and analyzed in general compliance with Centers for Disease Control and Prevention guidelines for leukocyte immunophenotyping of HIV-infected persons [6].

Peripheral blood lymphocytes were stained with combinations of monoclonal antibodies (MAbs), fixed, and analyzed within 12 h of staining. Isotype-matched MAbs served as controls for fluorescence marker settings and identification of nonspecific staining. All MAbs were directly conjugated to either fluorescein isothiocyanate (FITC), phycoerythrin (PE), or to a tandem dye, phycoerythrin–cyanine 5 (PE-Cy5). The following MAbs were used: CD3-PE-Cy5 (Pan-T cells; Immunotech, Westbrook, ME); MsIgG1-FITC, $MsIgG_{2a}$-PE, $MsIgG_1$–Cy5 (isotype control; Immunotech);

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Informed consent was obtained from all patients, and the research protocol was approved by the Joint Committee on Clinical Investigation of Johns Hopkins Hospital and School of Medicine.

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CD45-FITC (lymphocytes, monocytes; Becton Dickinson, San Jose, CA); CD14-PE (monocytes; Becton Dickinson); CD4-FITC (helper T cell; Becton Dickinson or Olympus, Lake Success, NY); CD8-PE (cytotoxic/suppressor T cell; Becton Dickinson or Olympus); CD28-FITC, CD28-PE (costimulatory molecule; Immunotech); CD45RA-FITC (naive T cell; Becton Dickinson); $CD16^{+}CD56^{+}$-PE (NK cell; Immunotech); and LFA-1–FITC (accessory, adhesion molecule; Immunotech).

Two- and three-color flow cytometry was done using Becton Dickinson FACStar Plus and FACStar Plus Research Software (LYSIS II). Flow cytometer optics were aligned and calibrated daily with Biosure chick red blood cells (Riese, San Jose, CA). For each sample, 15,000 ungated events were collected, and the lymphocyte population was selected by backgating. Quadrant analysis cursors were set from the isotype control sample so that >98% of the gated cells were double negative. These same quadrant settings were used for subsequent antibody combinations. The raw subset percentage value was corrected for nonlymphocyte events within the gate [6].

Raw data were analyzed with statistical software (SAS Institute, Cary, NC). For many analyses, all patients with CMV retinitis were considered a single group. Categorical characteristics were compared between groups by use of Fisher's exact test. Group means were compared using Student's *t* test. Median and interquartile values were used to describe lymphocyte subset ranges, which were not normally distributed. The percent counts and absolute counts of cell surface markers were compared using the Wilcoxon rank sum test. Multivariate regression analysis was done to adjust for potentially confounding factors. Unadjusted *P* values are given.

Results

One hundred five HIV-infected persons with $CD4^{+}$ cell counts $<0.050 \times 10^9$/L were recruited prospectively, of whom 49 had CMV retinitis (patients) and 56 had HIV-infection without CMV retinitis (controls).

Demographics for the patients and controls are compared in table 1. Of the 49 patients, 22 were receiving anti-CMV treatment (ganciclovir, foscarnet, or cidofovir); 27 had newly diagnosed CMV retinitis and were not receiving treatment at the time of blood sampling. Subgroup analyses showed no significant statistical differences in demographic factors and medical histories, except for granulocyte colony–stimulating factor (G-CSF) use, for treated and untreated patients, so they were analyzed as a single group. Patients were slightly older than controls, but the 2 groups did not differ significantly in their use of acyclovir, prednisone, or cytotoxic drugs; however, fewer patients were receiving antiretroviral drugs than were controls ($P = .049$), and more patients than controls were treated with G-CSF ($P < .001$). In addition, more patients than controls had a history of Kaposi's sarcoma ($P = .019$) and other opportunistic infections (i.e., *Pneumocystis carinii* pneumonia and cryptococcal and *Mycobacterium avium-intracellulare* complex infections; $P = .030$).

Lymphocyte subset results, comparing patients and controls, are listed in table 2. The median absolute lymphocyte count was significantly lower in the patient group than in the control group (0.517×10^9/L vs. 0.610×10^9/L, $P = .018$). However, both groups had a similar $CD4^{+}$ cell percent (0.81×10^9/L vs. 1.1×10^9/L, $P = .380$) and similar median absolute $CD4^{+}$ cell counts (both $<0.010 \times 10^9$/L, $P = .069$).

Compared with $CD8^{+}$ and $CD45RA^{+}$ cell counts for controls, those for patients were lower ($CD8^{+}$: $P <.001$; $CD45RA^{+}$: $P < .001$). $CD8^{+}CD45RA^{+}$ cell counts were also significantly lower in patients than controls ($P <.001$). Three-color analysis determined that most $CD3^{+}CD45RA^{+}$ cells (i.e., $CD45RA^{+}$ cells) were also $CD8^{+}CD45RA^{+}$ cells.

CD28 antigen expression was significantly lower for $CD3^{+}$ ($P = <.001$) and $CD8^{+}$ ($P = <.001$) cell subsets in patients than in controls. Three-color analysis determined that most of the $CD8^{+}CD28^{+}$ cells were also $CD3^{+}CD8^{+}CD28^{+}$ cells. T cells expressing LFA-1 were significantly lower in patients than controls ($P < .001$). Although the percentage of $CD3^{-}56^{+}CD16^{+}$ cells (NK cells) was higher in patients than controls, the absolute counts were similar.

A subgroup analysis was done to determine the effect of treatment with G-CSF. The absolute number of lymphocytes

Table 1. Demographics and medical histories for HIV-infected subjects with (patients) or without (controls) CMV retinitis.

Feature	Patients	Controls	P*
No. of patients	49	56	
Race			.051
White	61	41	
Black	39	59	
Gender			1.000
Male	86	88	
Female	14	12	
Risk factor			.190
MSM	65	46	
IDU	21	23	
MSM and IDU	0	7	
Heterosexual	12	20	
Blood product use	2	4	
Age (years), mean ± SD	40.3 ± 8.4	36.8 ± 6.5	.016
Antiretroviral drug use			.049
Yes	31	50	
No	69	50	
G-CSF			<.001
Yes	29	4	
No	71	96	
Kaposi's sarcoma			.019
Yes	22	5	
No	78	95	
History of other OI			.030
Yes	71	50	
No	29	50	

NOTE. Data are % unless otherwise indicated. MSM = men having sex with men; IDU = injection drug use; G-CSF = granulocyte colony-stimulating factor; OI = opportunistic infections (defined as *Pneumocystis carinii* pneumonia and cryptococcal or *Mycobacterium avium-intracellulare* complex infections).

* Determined by use of Fisher's exact test and Student's *t* test.

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Table 2. Percent and absolute lymphocyte counts for HIV-infected subjects with (patients) or without (controls) CMV retinitis.

	Patients				Controls	
Surface marker	$CD4^+$ cell % ($\times 10^9$/L)	P*	Absolute no. lymphocytes ($\times 10^9$/L)	P*	$CD4^+$ cell % ($\times 10^9$/L)	Absolute no. lymphocytes ($\times 10^9$/L)
$CD3^+CD8^+$	39.50 (19.13, 49.90)	.006	0.152 (0.075, 0.308)	<.001	49.62 (34.76, 60.66)	0.296 (0.163, 0.490)
$CD3^+CD45RA^-$	19.43 (9.16, 32.25)	.001	0.082 (0.036, 0.191)	<.001	34.23 (23.51, 43.68)	0.211 (0.083, 0.367)
$CD8^+CD45RA^+$	16.53 (8.50, 34.21)	<.001	0.078 (0.035, 0.138)	<.001	30.38 (22.48, 39.12)	0.203 (0.095, 0.341)
$CD3^+CD28^+$	13.38 (5.08, 24.22)	<.001	0.048 (0.019, 0.144)	<.001	23.47 (16.54, 36.03)	0.143 (0.093, 0.262)
$CD8^+CD28^-$	6.82 (1.91, 15.40)	<.001	0.023 (0.010, 0.090)	<.001	15.08 (9.18, 26.14)	0.088 (0.049, 0.185)
$CD8^+CD28^-$	25.65 (17.02, 40.36)	.418	0.120 (0.063, 0.211)	.014	29.29 (19.86, 45.59)	0.200 (0.113, 0.323)
$CD3^+LFA\text{-}1^+$	51.46 (30.88, 62.86)	.004	0.238 (0.120, 0.372)	<.001	63.92 (51.10, 73.11)	0.400 (0.202, 0.594)
$CD3^-CD56^+CD16^+$ (NK cells)	12.45 (6.16, 22.35)	.021	0.063 (0.024, 0.108)	.763	7.38 (3.69, 16.00)	0.054 (0.024, 0.099)

NOTE. Data are medians; numbers in parentheses are 25th, 75th percentile values.
* Compared with controls; determined by use of Wilcoxon rank sum test.

was significantly higher in patients receiving G-CSF compared with those not receiving G-CSF (median: 0.617×10^9/L vs. 0.384×10^9/L, $P = .023$). The absolute number of $CD8^+$ cells in patients receiving G-CSF versus those not receiving G-CSF was also significantly higher (0.308×10^9/L vs. 0.138×10^9/L, $P = .038$), as was the absolute number of $CD28^+$ cells (0.136×10^9/L vs. 0.033×10^9/L, $P = .010$) and $CD8^+CD28^+$ cells (0.08×10^9/L vs. 0.019×10^9/L, $P = .027$). T cells counts were also significantly higher in G-CSF–treated patients. No significant group differences were found for other mononuclear subsets analyzed.

Multivariate regression analysis showed that the differences in cell surface markers between patients and controls remained after adjusting for age, race, and antiretroviral use. The only factor that influenced the cell surface marker results was G-CSF use, which actually reduced the differences between patients and controls.

Discussion

HIV-infected patients with CMV retinitis and controls without CMV retinitis were matched for $CD4^+$ cells by selecting subjects with $CD4^+$ cells $<0.050 \times 10^9$/L.

Patients were more likely than controls to be white and older and to have a history of another opportunistic infection, and they were less likely to be using antiretroviral drugs and more likely to be using G-CSF. However, regression analysis showed that age, race, and antiretroviral drug use did not alter the cell surface marker results, and G-CSF use reduced the differences in some mononuclear subset measurements between patients and controls. Subgroup analysis also showed no significant differences in the demographics or medical histories of treated and untreated patients, except for the greater use of G-CSF in the treated group.

Cell transfer studies in CMV-infected murine models suggest that $CD8^+$ cytotoxic T lymphocytes are protective for CMV disease [7]. Our group of patients had significantly lower $CD8^+$ cell counts compared with those for the control group, and the majority of these cells were naive ($CD45RA^+$). Fiala et al. [8] reported that the risk for CMV disease increases when $CD8^+$ cell counts decline to $<0.500 \times 10^9$/L. This finding was corroborated by Lowder et al. [9] in a retrospective study of CMV retinitis in patients with AIDS. These findings underscore the importance of $CD8^+$ cytotoxic T lymphocytes in the prevention of CMV retinitis.

It has been suggested that switching from $CD45RA^+$ (naive) to $CD45RO^+$ (memory) cells is an important mechanism of T cell activation in response to CMV infection. In this study, patients with CMV retinitis had lower numbers of naive cells than did the control group, suggesting that the switching of naive cells to memory cells may be inadequate to prevent CMV disease in HIV-infected patients.

CD28 antigen provides the necessary second signal for T cell activation, proliferation, and interleukin-2 production. Brinchmann et al. [5] demonstrated that in HIV-infected patients, the ability to proliferate and secrete interleukin-2 in response to T cell receptor activation was restricted to the $CD8^+CD28^+$ cell subset. In the current study, we found a significant decrease in T cell–expressed CD28 antigen in the HIV-infected patients with CMV retinitis, compared with HIV-

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infected controls. This loss of CD28 antigen was mainly observed in the $CD8^+$ population of T cells, suggesting that loss of the costimulatory molecule may result in a less effective cytotoxic/suppressor response to CMV-infected cells. The greater loss of CD28 antigen expression might also explain the poorer proliferative responses of T cells isolated from HIV-infected persons with CMV retinitis compared with responses of T cells from HIV-infected persons without CMV retinitis [10]. Factors other than CD28 antigen expression may also contribute to the poorer lymphoproliferative responses observed in vitro. For example, ganciclovir, acyclovir, and zidovudine each have been reported to inhibit lymphocyte proliferation in vitro [11]. Furthermore, CMV infection itself has been reported to have immunosuppressive effects, manifested by a decreased lymphoproliferative response after mitogen stimulation and by decreased cytolytic T cell and NK cell activity. However, lymphocytes are usually nonpermissive for CMV infection, so a direct immunosuppressive effect of the virus at the T cell level is unlikely.

Interactions between LFA-1 and intercellular adhesion molecule 1 contribute to the activation of the T lymphocyte and can augment cytotoxicity of virus-specific $CD8^+$ cells [12]. In the current study, HIV-infected patients with CMV retinitis had significantly lower levels of $CD3^+LFA\text{-}1^+$ cells compared with levels for the HIV-infected controls. Loss of LFA-1 could further contribute to immunodeficiency and, thus, to an increased risk of CMV retinitis.

Animal and clinical studies have shown that NK cells play a role in CMV disease, though they generally delay but do not prevent severe disease and death [13]. In our study, there were no significant differences in the absolute numbers of NK cells in subjects with or without CMV retinitis, suggesting that the cells have a less important role in the prevention of CMV disease in HIV-infected persons with progressive immunosuppression.

A subgroup analysis of T cell subsets was done to study the effects of G-CSF in HIV-infected patients with CMV retinitis. It has been previously reported that there is a disproportionate increase in $CD8^+$ cells during treatment with G-CSF [14]. In our study, we found significant increases in the absolute lymphocyte and $CD8^+$ cell counts but no effect on the $CD4^+$ cell count. The $CD8^+CD28^+$ cell numbers also increased in patients treated with G-CSF. The G-CSF–initiated increase in cells bearing costimulatory molecule CD28 is consistent with the reported increase in lymphoproliferative responses to phytohemagglutinin of T cells from HIV-infected patients treated with G-CSF compared with those from untreated patients [14]. This effect of G-CSF on T cell subsets tended to minimize the differences between subjects with and without CMV retinitis.

Whether the observed changes in T cell subsets in HIV-infected patients with CMV retinitis represent a cause or effect of CMV disease cannot be determined from this study. However, in light of previously published observations and the observations from our study, one plausible hypothesis is that a loss of $CD8^+$ cytotoxic T cells with down-regulation of CD28 and LFA-1 antigens (a change that leads to an ineffective cytotoxic response to virus-infected cells) contributes to an increased risk for CMV retinitis. Screening of HIV-infected patients with $CD4^+$ cell counts $<0.050 \times 10^9$/L, $CD8^+$ cell counts $<0.500 \times 10^9$/L, and low numbers of $CD8^+CD28^+$ and $CD8^+LFA\text{-}1$ cells might better identify patients at risk of CMV retinitis. Such patients may benefit from primary prophylaxis for CMV disease.

Finally, successful adoptive immunotherapy for human viral infections requires an understanding of immune mechanisms essential for providing the host with a protective response. The observed changes in the cytotoxic cell subset profile in AIDS and CMV retinitis may also help to develop more virus-specific T cell subsets for adoptive therapy for CMV disease [15].

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Human Immunodeficiency Virus RNA Levels in US Adults: A Comparison Based upon Race and Ethnicity

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Volunteers in a natural history study of human immunodeficiency virus type 1 (HIV-1) at two military medical centers were studied to determine whether plasma HIV-1 RNA levels differ among racial and ethnic groups of US adults infected with HIV-1. Cross-sectional analyses of plasma HIV-1 RNA and CD4 cell counts were done using demographic and clinical data collected during study visits. Age, gender, CD4 cell count, seroconversion status, and use of antiretroviral therapy were studied in 545 military members (46% white, 49% black, and 6% Hispanic). No association was found between HIV-1 RNA levels and race or ethnicity among infected adults for whom access to care and socioeconomic status were not confounding factors.

Minority groups within the United States are affected disproportionately by the human immunodeficiency virus type 1 (HIV-1) epidemic. In the most recent national report of leading causes of death, the ratio of age-adjusted death rates related to HIV-1 infection is 4:1 for black versus white persons [1]. Since 1991, AIDS has been the leading cause of death for black and Hispanic men and black women 25–44 years old [2]. A number of studies have found that survival time for persons with AIDS differs among racial groups [3], and a recent article suggests that CD4 cell count decline varies with race [4]. However, such comparisons are generally confounded by socioeconomic factors and unequal access to health care. Analyses of both hemophilia and homosexual cohorts have shown that the level of HIV-1 RNA circulating in the serum or plasma is a predictor of time to development of AIDS and to death [5, 6]. Whether virus load (circulating RNA level) differs among racial and ethnic groups is unknown, and virus load may affect the rate of disease progression.

The factors confounding most comparisons of racial and ethnic groups in the United States are equalized within the military system. Quality health care is accessible to all members. More specifically, HIV-1 screening and patient management are carried out according to standardized policies [7]. A long-term study of the natural history of HIV-1 infection established by the US Department of Defense (DoD) has enrolled approximately equal numbers of white and black volunteers and a smaller number of Hispanic volunteers [8]. As part of this study, HIV-1 RNA quantitation in plasma was initiated in 1995 through a single central laboratory. Herein we report on an analysis that was based on results of the initial year of testing. The analysis addresses the hypothesis that differences in HIV-1 virus load may be associated with race.

Materials and Methods

Study participants were recruited from among volunteers enrolled in a DoD protocol for investigating the natural history of HIV-1 infection in members of the US military. Inclusion criteria

Written informed consent was obtained from all study subjects, and human experimentation guidelines of the US Department of Defense, Walter Reed Army Medical Center, and the National Naval Medical Center were followed in the conduct of this research.

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Research/Recherche

Efficiency of EPI cluster sampling for assessing diarrhoea and dysentery prevalence

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This study examines the efficiency of EPI cluster sampling in assessing the prevalence of diarrhoea and dysentery. A computer was used to simulate fieldwork carried out by a survey taker. The bias and variance of prevalence estimates obtained using EPI cluster sampling were compared with those obtained using simple random sampling and cluster (stratified random) sampling. Efficiency ratios, calculated as the mean square error divided by total distance travelled, were used to compare EPI cluster sampling to simple random sampling and standard cluster sampling. EPI cluster sampling may be an appropriate low-cost tool for monitoring trends in the prevalence of diarrhoea and dysentery over time. However, it should be used with caution when estimating the prevalence of diarrhoea at a single point in time because of the bias associated with this cluster sampling method.

Introduction

Diarrhoea is one of the major causes of morbidity and mortality among children in developing countries. It has been estimated that over 12 000 persons die each day from diarrhoeal disease in developing countries (*1, 2*). Diarrhoea has been shown to affect the growth of children and is an important cause of malnutrition which can adversely affect child mortality (*3, 4*). The infectious nature of diarrhoea suggests that faecal contamination of water and food contributes to its transmission (*5*). For example, a contaminated source of water, if serving several families, can cause a cluster of illness.

The prevalence of diarrhoea and dysentery in a community can be estimated through the use of a sample survey. Depending on the method used, a sample survey can give an accurate overview of the disease process within a community. For infectious diseases such as diarrhoea and dysentery, a properly designed and conducted sample survey can provide an epidemiological snapshot of the disease. A diarrhoeal disease survey may also be used to assess the nutritional status of a community, since children affected with diarrhoea and dysentery are often nutritionally deficient. A survey can also establish baseline information prior to an intervention. Repeated over time, cross-sectional surveys are useful surveillance tools for monitoring changes in a population.

There are a number of sampling strategies that can be used to ascertain the prevalence of diarrhoea and dysentery in a community. Theoretically the simplest, but often the most difficult to carry out, is simple random sampling (SRS). SRS requires each member of the population to have a known and equal probability of selection. The list from which the sample is drawn is assumed to include every eligible member. The compilation of this list, often called the sampling frame, is difficult and sometimes impossible, even in situations with large amounts of resources. In developing countries, acquisition of the sampling frame can be especially difficult because of inaccuracies in the existing census, to which are added high migration and birth rates and severely limited resources.

An additional difficulty with SRS which makes it impractical in most situations is that subjects selected in the sample can be geographically spread out. Visiting these subjects can divert resources from other areas of the survey or even other health pro-

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grammes. Inaccessible areas containing only one or two subjects may need to be visited. Consequently, SRS is infrequently used.

A method that eliminates many of the logistical problems of SRS is cluster sampling. The primary sampling units are clusters, often defined so as to be convenient for the researcher (*6*). For example, clusters may be city blocks if the survey is conducted in an urban setting, or villages if the survey is conducted in rural areas. For cluster sampling, a complete list of clusters is necessary, which is usually much easier to obtain than the sort of list required for SRS. However, a complete list of individual elements is necessary for selected clusters. SRS is often used for sampling within clusters. If clusters are "large", the amount of resources necessary to list their elements can still be substantial.

Thus, a simplified low-cost methodology (EPI cluster sampling) was developed by the WHO Expanded Programme on Immunization (EPI) and has been used extensively (*7*). Typically, EPI cluster surveys have consisted of 30 clusters of 7 children each (for immunization coverage surveys) (*8*). However, the method has been modified and adapted for other purposes: 30 clusters of 14 children have been used for surveys of immunization coverage, oral rehydration salt availability, and breast-feeding and dietary information (*9*); 30 clusters of 68 children were used in Guinea to monitor selective components of primary health care (*10*); 30 clusters of 70 children were used for surveying neonatal tetanus mortality in Kenya (*11*); 30 clusters of 215, for assessing morbidity and mortality due to diarrhoea in the Central African Republic (*12*); 19 clusters of 10–13 children each, in the Philippines (*13*); and 45 clusters of 8 children, for breast-feeding practices (*14*).

Standard cluster sampling uses SRS for sampling within clusters; however, EPI cluster sampling uses a convenient sampling procedure instead of probability-based SRS for second-stage sampling. Thus, although EPI cluster sampling is easier and less costly, the bias and variance of the estimates obtained can increase.

The present study investigates the cost of conducting EPI cluster sample surveys (expressed as distance travelled to visit the sample subjects) and the bias and variance of the estimates derived with this sampling design.

Materials and methods

Data were derived from a survey of preschool-child morbidity that was carried out prior to a randomized vitamin A supplementation trial in the Terai district of Sarlahi, Nepal (*15, 16*). Communities enrolled in the trial were mapped, and each house was numbered. We used data from a subset of 40 wards in 25 villages where morbidity histories, anthropometric measurements, and ocular health indicators were collected at the beginning of and at regular intervals during the trial. The 40 wards were selected with probability proportional to size from among 261 wards in the trial.

All children under 5 years of age were identified by a house-to-house census and enrolled in the study. At the outset, respondents were queried to determine if enrolled children had either a 7-day history of diarrhoea (defined as four or more loose, watery stools per day) or dysentery (defined as the presence of blood in the stool regardless of whether diarrhoea was present). Other information on health and nutritional status was also collected. Interviews were conducted by persons with at least 10 years of formal education, but without medical training. Local terms for symptoms were used, which had been previously identified through focus-group discussions within the communities. Children with symptoms on one or more days were considered to be ill. Children not under direct observation by a parent for the past week were considered to have missing morbidity data.

Maps of each of the wards were established for the census. Each house was identified on the maps with the same identifier as used in the database. Although not drawn to scale, the maps were the means of locating households for subsequent visits. The quality of the data collected has been described (*17*). The study population consisted of 4297 children who participated in the baseline survey and had a history of morbidity.

Three different sampling schemes were simulated: SRS, stratified random sampling, and EPI cluster sampling. Modified versions of the EPI cluster sampling scheme were also simulated. The SRS method randomly selected a sample of fixed size from the total population of eligible children, regardless of ward of residence. For stratified random sampling, a fixed number of children in each of the 40 wards was randomly selected. For EPI cluster sampling a fixed number of children in each ward was selected using an EPI-recommended sampling strategy. All simulations were carried out using the established maps.

Like cluster sampling, EPI cluster sampling often uses probability-proportional-to-size (PPS) sampling to select clusters. However, at the second stage, EPI cluster sampling selects a starting household in each community by locating the ward's centre, randomly selecting a direction, and randomly selecting a house from a list of all houses falling along the line drawn from the ward centre to the

periphery in the chosen direction. All eligible children in the selected households are included. If additional children are required, the closest house to the right is visited, until the required number of children are sampled. If a household has more children than required for a given sample size, all children in the household are nevertheless sampled. A version of EPI cluster sampling that has the effect of spreading out the sampled children across the community by visiting the *n*th nearest house to the right was also simulated.

A total of 1000 simulations were performed for each sample size investigated. For SRS, sample sizes of 280, 400, 600, 800, and 1000 were simulated. For stratified random sampling, sample sizes of 7, 10, 15, 20, and 25 children per ward were simulated for all 40 wards, resulting in sample sizes of 280, 400, 600, 800, and 1000. For EPI cluster sampling, a minimum of 7, 10, 15, 20, and 25 children per ward were sampled in each of the 40 wards, resulting in sample sizes of approximately 280, 400, 600, 800, and 1000. Sample sizes were selected to provide a range of estimates of bias and variance for each sampling method and to allow comparisons with previous computer simulations of EPI cluster sampling (*18*, *19*).

Simulations required computer maps representing the locations of households and allowing calculations to be made of the distance travelled in wards for each sampling method. A digitizing tablet and MapInfo (MapInfo Corporation, NY, USA) geographic-information-system software were used to enter household locations, which were referenced to an arbitrary point of origin. The centre of each ward was calculated by taking the average of the coordinates of the households within a ward. For SRS and stratified random sampling, a Turbo Pascal (Borland Corporation, CA, USA) program determined, based on the list of selected children, the child living closest to the ward centre. From this initial household, the nearest household containing a selected child was determined and visited. The software was used to keep track of disease status and the total distance travelled. "Visiting" the nearest household containing a selected child was repeated until all selected children were included. Since the sampling unit for SRS and stratified random sampling was the eligible child, only households with eligible children were "visited".

For EPI cluster sampling, the sampling unit was the household. A household was selected at random from a list of all households falling within a path of a defined width from the ward centre in a randomly chosen direction. The house was then examined to determine if a child of eligible age was living there. Subsequently, the nearest (or *n*th nearest) household to the right was "visited", and the steps repeated until the desired number of children was obtained. The prevalence of diarrhoea and dysentery and the total distance travelled, including the distance from the centre of the ward to the starting household, were determined. The total distance travelled also included "visiting" households with no eligible children.

The "true" disease prevalence was considered to be the number of children with diarrhoea (or dysentery) as determined by the census, divided by the total number of enrolled children. Disease prevalence estimates from stratified random sampling and EPI cluster sampling were weighted according to the number of eligible children in each ward, in order to obtain estimates that could be compared with "true" prevalence. In a survey in which the primary sampling units (wards) would have been PPS selected, an unweighted prevalence estimate for the stratified random sample or the EPI cluster sample would have been appropriate because of the fixed sample size in each cluster. A prevalence estimate was obtained for each of the 1000 simulations run for each method. The mean prevalence of the 1000 simulations was then compared with true prevalence to estimate the bias of each method. Variance was obtained from the distribution of the 1000 prevalence estimates.

The effect of sampling design (design effect) on the variance of estimates obtained with EPI cluster sampling was calculated by dividing the estimate variance for EPI cluster sampling by the estimate variances for SRS and stratified random sampling. Thus, two different design effects were calculated. One answers the question "How much does estimate variance increase if cluster sampling instead of SRS is used to assess the prevalence of diarrhoea and dysentery?"; the second answers the question "For the wards selected using PPS sampling at the first stage, how much does estimate variance increase using EPI cluster sampling instead of SRS for the second sampling stage?" Mean square errors (MSE) were calculated from the bias and variance estimates derived from stratified random and EPI cluster sampling by adding the square of bias to variance. MSE represents the overall error of prevalence estimates under different sampling designs.

Estimates of distance travelled were compared for EPI cluster sampling and stratified random sampling for each simulation run. Distance was compared ward by ward because ward maps were not drawn to a common scale and mapping distortion was variable. Median distance was calculated for each ward for each sampling method. A distance ratio was calculated for each ward by dividing the median distance travelled for EPI cluster sampling

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by median distance travelled for stratified random sampling. A ratio greater than 1 indicates that EPI cluster sampling required a greater travel distance.

Maps of households visited for EPI cluster sampling (Fig. 1) and stratified random sampling (Fig. 2) are shown for ward 1.2. In Fig. 1, of the eight houses visited during EPI cluster sampling simulation, eligible children were found in only three. The distance travelled is greater for children selected at random (Fig. 2) than for those selected using the EPI sampling method (Fig. 1).

MSE measures the total error of sample estimates and distance can be used as an indicator of cost. To compare two designs (*a* & *b*), one can use the efficiency ratio (*20*):

$$\frac{Cost_a \times MSE_a}{Cost_b \times MSE_b}$$

By these criteria, a ratio greater than 1 implies design *b* is preferred, i.e. it has either a smaller cost per unit MSE or a smaller MSE per unit cost. The product of the cost and MSE of estimates obtained from EPI cluster sampling was compared to that of stratified random sampling for the same sample size.

Limitations

Distance travelled can provide only an approximate indication of the cost of conducting a survey. Other costs may include personnel, training, and materials. However, for comparison across sample designs, distance travelled should provide reasonably accurate estimates. However, if the cost of obtaining an accurate sampling frame is considered, SRS may be far more costly.

Distance was calculated as a straight line between two points. Roads, rivers, and other physical obstacles were not taken into consideration. Again, however, all simulations were subject to the same constraints, so comparison of designs should not have been seriously affected.

Finally, a field worker cannot be perfectly modelled using a computer. For example, the role of individual subjectivity in household selection cannot be simulated. As the selection algorithm used in the simulations is completely objective and consistent, the simulations represent an "ideal" that may be more or less close to the real situation.

Results

A total of 4297 (90.2%) of 4765 eligible children lived in 54.7% of the 4920 households in the 40 wards under study. The number of eligible children in each ward ranged from 13 to 284, whereas the number of households ranged from 31 to 315. The "true" prevalence of diarrhoea was 13.8%, and the prevalence of dysentery 4.4%, among the 4297 children surveyed.

Fig. 1. **Map of ward 1.2 showing the households visited (starting with household 27) for EPI cluster sampling** (sample size 7, next nearest household to the right visited).

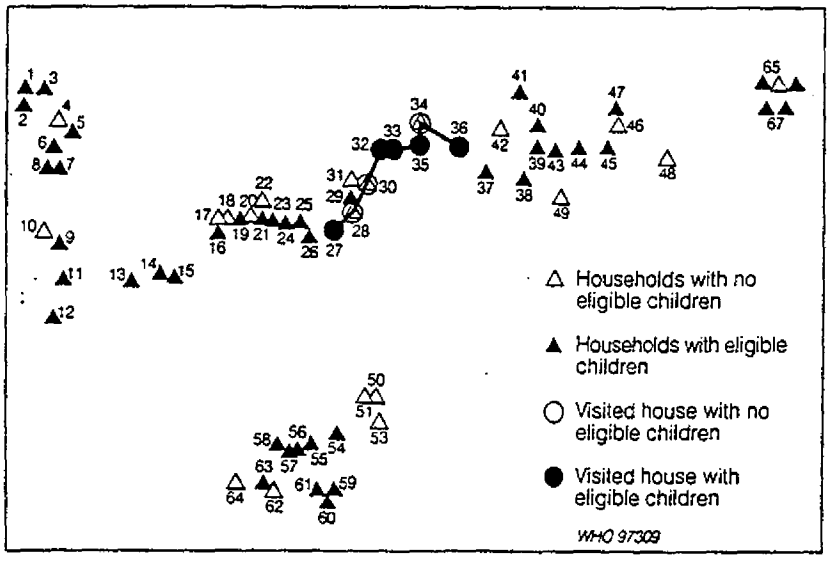
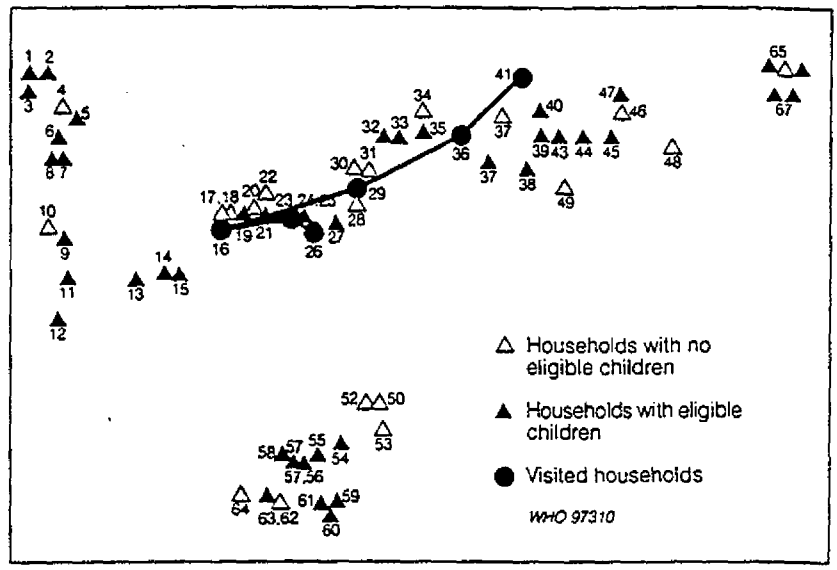


Fig. 2. **Map of ward 1.2 showing households visited (starting with household 26) for stratified random sampling** (sample size 7).



Estimates based on simulations ranged from 13.75% (simple random sampling, sample size 10) to 16.45% (EPI cluster sampling, sample size 7, 4th nearest house to the right selected) for diarrhoea (Table 1) and 3.85% (EPI cluster sampling, cluster size 25, nearest house to the right selected) to 5.4% (EPI cluster sampling, cluster size 7, 3rd nearest house to the right selected) for dysentery (Table 2). For diarrhoea, EPI cluster sampling always resulted in higher prevalence than "true" prevalence.

Bias, calculated as the difference between true and sample prevalence, was very small for stratified random sampling. However, bias from EPI cluster sampling ranged from 0.36% to 2.65% (diarrhoea) and −0.55% to 1.00% (dysentery) (Fig. 3 & 4). EPI-derived estimates for diarrhoea prevalence showed positive bias (EPI cluster sampling consistently overestimated diarrhoea prevalence). Bias for EPI derived dysentery prevalence estimates was either positive or negative, depending on the number of houses to the right that were skipped. The typical EPI procedure of selecting the next nearest household consistently underestimated the prevalence of dysentery (negative bias) regardless of sample size.

Estimate variances for EPI cluster sampling were compared to estimate variances for SRS to estimate the design effect of EPI cluster sampling. This estimates the overall increase or decrease in estimate

Table 1: **Prevalence estimates for diarrhoea obtained from EPI cluster sampling and simple random sampling (SRS)**

Sample size	True prevalence	EPI (1)[a]		EPI (2)		EPI (3)		EPI (4)		EPI (5)		SRS	
		Mean	SD[b]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
7	13.80	15.76	2.31	16.00	2.34	15.85	2.47	16.45	2.28	15.58	2.09	13.80	2.53
10	13.80	15.79	1.77	15.68	1.96	16.11	1.98	15.46	1.76	15.32	1.78	13.75	2.14
15	13.80	15.06	1.32	14.95	1.49	15.91	1.37	14.63	1.23	14.35	1.24	13.78	1.55
20	13.80	14.94	1.08	14.71	1.25	15.27	1.04	14.23	1.00	14.16	1.02	13.82	1.42
25	13.80	15.35	0.80	14.28	0.99	15.01	0.80	14.57	0.89	14.27	0.86	13.81	1.23

[a] Figures in parentheses are the number of households to the right that were skipped.
[b] SD = standard deviation.

Table 2: **Prevalence estimates for dysentery obtained from EPI cluster sampling and simple random sampling (SRS)**

Sample size	True prevalence	EPI (1)[a]		EPI (2)		EPI (3)		EPI (4)		EPI (5)		SRS	
		Mean	SD[b]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
7	4.40	4.02	1.26	5.07	1.58	5.40	1.49	4.82	1.39	4.99	1.46	4.44	1.49
10	4.40	4.05	1.00	4.81	1.32	5.13	1.17	4.47	1.04	4.58	1.08	4.45	1.23
15	4.40	4.09	0.76	4.78	0.88	4.71	0.75	4.02	0.73	4.17	0.73	4.41	0.98
20	4.40	3.91	0.63	4.49	0.66	4.49	0.62	4.06	0.58	4.02	0.61	4.40	0.82
25	4.40	3.85	0.50	4.13	0.55	4.30	0.49	3.90	0.44	3.97	0.55	4.42	0.72

[a] Figures in parentheses are the number of the households to the right that were skipped.
[b] SD = standard deviation.

variance due to use of the EPI cluster sampling design compared with the random selection of children from the entire census list (Table 3). The design effect for estimates of diarrhoea prevalence ranged from 0.47 to 1.52 for EPI cluster sampling versus SRS. The value 0.47 was obtained from EPI cluster sampling with a sample size of 25 when the nearest house to the right was visited. The value 1.52 was obtained from EPI cluster sampling with a sample size of 7 when the 3rd nearest house to the right was visited. For estimates of dysentery prevalence, the design effect ranged from 0.46 (sample size 25, 4th nearest house to the right) to 1.76 (sample size 7, 2nd nearest house to the right) (Table 3). In general, larger sample sizes resulted in smaller design effects. The number of houses to the right that were skipped did not influence the design effect.

The comparison of estimate variances from EPI cluster sampling with those from stratified random sampling estimates the impact of using EPI cluster sampling instead of random sampling at the second sampling stage. This gives an estimate of that portion of the overall increase or decrease in variance associated with EPI cluster sampling that is due solely to the second sampling stage. The design effect for estimates of diarrhoea prevalence ranged from 0.42 (sample size 25, nearest house or 3rd nearest house to the right) to 0.95 (sample size 7, 3rd nearest house to the right) (Table 3). For estimates of dysentery prevalence, the design effect ranged from 0.37 (sample size 25, 4th nearest house to the right) to 1.15 (sample size 10, 2nd nearest house to the right) (Table 3). These design effects from EPI cluster sampling versus stratified random sampling were

Fig. 3. **Prevalence estimates for diarrhoea, by number of households to the right skipped and sample size.** (SRS = simple random sampling, EPI(n) = n households to the right skipped).

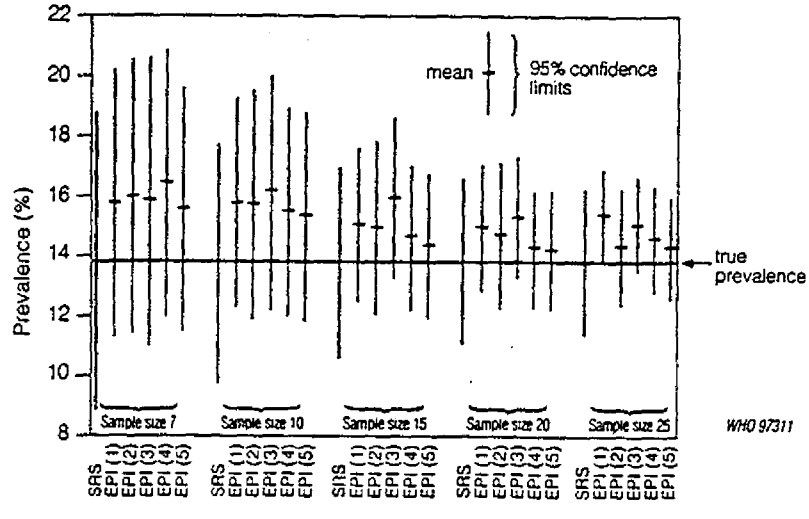


Fig. 4. **Prevalence estimates for dysentery, by number of households to the right skipped and sample size.** (SRS = simple random sampling, EPI(n) = n households to the right skipped).

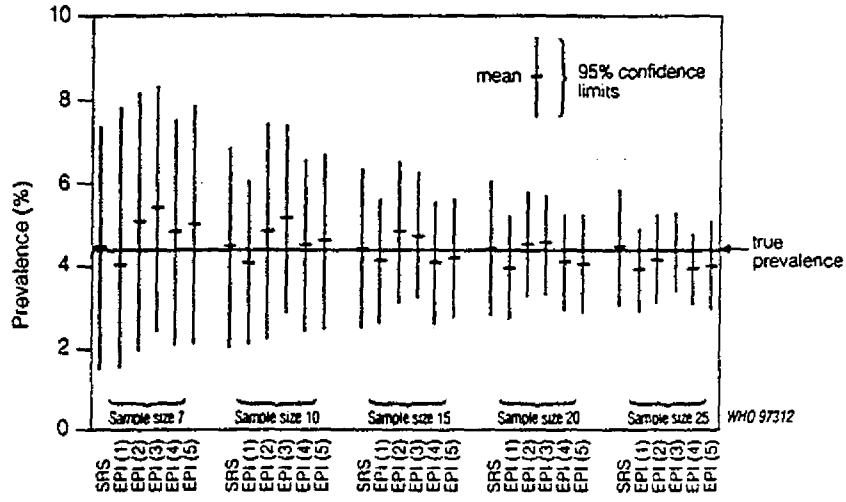


Table 3: **The design effects of EPI cluster sampling**

Design	Diarrhoea[a]	Diarrhoea[b]	Dysentery[a]	Dysentery[b]
EPI (1, 7)[c]	1.33	0.83	1.12	0.72
EPI (1, 10)	1.01	0.73	0.98	0.65
EPI (1, 15)	1.02	0.64	0.90	0.59
EPI (1, 20)	0.98	0.58	0.95	0.60
EPI (1, 25)	0.47	0.42	0.61	0.48
EPI (2, 7)	1.36	0.85	1.76	1.13
EPI (2, 10)	1.25	0.91	1.73	1.15
EPI (2, 15)	1.30	0.81	1.22	0.80
EPI (2, 20)	1.31	0.77	1.05	0.66
EPI (2, 25)	0.72	0.65	0.73	0.58
EPI (3, 7)	1.52	0.95	1.56	1.00
EPI (3, 10)	1.27	0.92	1.35	0.89
EPI (3, 15)	1.10	0.69	0.89	0.58
EPI (3, 20)	0.92	0.54	0.90	0.57
EPI (3, 25)	0.47	0.42	0.59	0.46
EPI (4, 7)	1.30	0.81	1.35	0.87
EPI (4, 10)	1.00	0.73	1.07	0.71
EPI (4, 15)	0.88	0.55	0.86	0.56
EPI (4, 20)	0.84	0.50	0.81	0.51
EPI (4, 25)	0.57	0.52	0.46	0.37
EPI (5, 7)	1.09	0.68	1.50	0.96
EPI (5, 10)	1.03	0.75	1.16	0.77
EPI (5, 15)	0.90	0.56	0.86	0.56
EPI (5, 20)	0.87	0.51	0.88	0.55
EPI (5, 25)	0.54	0.49	0.73	0.58

[a] Compared with simple random sampling.
[b] Compared with stratified random sampling.
[c] Figures in parentheses are, respectively, the number of households to the right that were skipped and the sample size.

all less than one, except for those associated with estimates of dysentery prevalence with sample sizes 7 or 10, (2nd nearest house to the right).

The ratio of median distances (cost) increased when either n increased (nth nearest house to the right) or sample size increased (Table 4). The median distance ratios were all less than one when either nearest neighbours or next nearest neighbours were visited, regardless of sample size. This meant that for EPI cluster sampling visiting either the nearest house or the second nearest house, the distance travelled was always less than distance travelled for stratified random sampling to obtain the same number of subjects. If either the 3rd, 4th, or 5th nearest household was visited, the distance ratio was greater than one for large sample sizes. In other words, by increasing n and cluster size, the distance travelled using EPI cluster sampling increased more than the distance travelled for similar sample sizes using stratified random sampling. The cost benefit of EPI cluster sampling (at least for small neighbourhoods within a village) is negated when households farther away than the 2nd nearest to the right are visited.

Efficiency ratios based on comparisons of the MSE and cost of prevalence estimates for diarrhoea obtained from EPI cluster sampling versus stratified random sampling increased with the sample size

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Table 4: **Median distance ratios of EPI cluster sampling compared with stratified random sampling**

	*n*th nearest household to the right sampled:				
Cluster size	1	2	3	4	5
7	0.39	0.53	0.64	0.72	0.82
10	0.42	0.58	0.68	0.80	0.92
15	0.49	0.62	0.80	1.01	1.07
20	0.55	0.77	0.92	1.28	1.33
25	0.65	0.95	1.13	1.47	1.62

when the nearest household to the right was visited (Table 5). The values ranged from 0.58 (sample size 7, nearest household to the right) to 1.97 (sample size 15, 3rd nearest household to the right). Efficiency ratios compare EPI cluster sampling and stratified random sampling, and an efficiency ratio less than one indicates that EPI cluster sampling is more efficient than stratified random sampling, considering both distance travelled and size of MSE. Similar results were obtained for estimates of dysentery prevalence: ratios ranged from 0.31 (sample size 7, nearest household to the right) to 1.51 (sample size 25, 5th nearest household to the right). The ranking of efficiency ratios on the basis of the nearness of households visited for a given cluster size was different for diarrhoea and dysentery, suggesting that the two diseases have different patterns of spatial distribution.

Table 5: **The ratios of the products of distance travelled and mean square error (efficiency ratios) for EPI cluster sampling versus stratified random sampling**

Design	Diarrhoea	Dysentery
EPI (1, 7)*	0.58	0.31
EPI (1, 10)	0.74	0.31
EPI (1, 15)	0.64	0.34
EPI (1, 20)	0.73	0.53
EPI (1, 25)	1.44	0.69
EPI (2, 7)	0.89	0.71
EPI (2, 10)	1.06	0.73
EPI (2, 15)	0.85	0.59
EPI (2, 20)	0.98	0.51
EPI (2, 25)	0.82	0.68
EPI (3, 7)	1.07	0.93
EPI (3, 10)	1.56	0.85
EPI (3, 15)	1.97	0.55
EPI (3, 20)	1.61	0.53
EPI (3, 25)	1.75	0.54
EPI (4, 7)	1.44	0.68
EPI (4, 10)	1.16	0.57
EPI (4, 15)	0.87	0.72
EPI (4, 20)	0.81	0.87
EPI (4, 25)	1.48	1.24
EPI (5, 7)	1.01	0.92
EPI (5, 10)	1.25	0.73
EPI (5, 15)	0.76	0.66
EPI (5, 20)	0.82	1.02
EPI (5, 25)	1.13	1.51

* Figures in parentheses are, respectively, the number of households to the right that were skipped and the sample size.

Discussion

EPI cluster sampling is often used when resources are limited, as it is easier and quicker to carry out than SRS or stratified random sampling. However, EPI cluster sampling can result in increased estimate bias and variance compared with SRS or stratified random sampling. In this study, EPI cluster sampling resulted in prevalence estimates for diarrhoea that were consistently larger than the prevalence as determined by census, regardless of sample size or the number of houses to the right that were skipped. Estimates for the prevalence of dysentery varied. Some showed positive bias and others negative, depending on sample size and the number of households skipped (Tables 1 & 2).

A possible explanation for the different bias patterns is that diarrhoeal disease may tend to be more concentrated in the centre of a village; as EPI cluster sampling tends to select starting households that are central rather than peripheral, diarrhoea prevalence may be overestimated. EPI cluster sampling may therefore introduce bias in prevalence estimates according to the actual pattern of disease distribution. Diarrhoea and dysentery were examined in this study because they are infectious diseases and may be transmitted by a single source of infection. In addition, children from the same neighbourhood often play together, which may add to infectious disease transmission and shape the resulting spatial distribution of disease.

On the basis of this study, we conclude that diarrhoea prevalence estimated with EPI cluster sampling would be an overestimate of the prevalence of diarrhoea. If the accuracy of a single survey is important, EPI cluster sampling may not be the right tool. However, if surveys are used to assess changes in prevalence over a period of time, EPI cluster sampling may be appropriate. EPI cluster sampling would in any case be less expensive and quicker to carry out than SRS or stratified random sampling.

For less common diseases such as dysentery, EPI cluster sampling may be appropriate for assessing community disease prevalence when either accuracy or the measurement of trends is important. No systematic bias due to EPI cluster sampling was found when estimating dysentery prevalence, and there was very little increase in variance associated

with EPI cluster sampling compared with stratified random sampling. However, spatial clustering can occur even with diseases of low prevalence, and EPI cluster sampling may in such a case produce biased estimates.

The spreading of sampled households over a larger geographical area by increasing n when selecting every nth household to the right should theoretically result in more heterogeneous samples and greater precision of estimates. It may also reduce their bias, since sampling from a larger area may minimize the influence of the "neighbourhood" effect (i.e., similarities in variables of interest between houses located near each other). Another way to obtain more heterogeneous samples is to interview only one child per household. This has the benefit of removing within-household similarities from the sample (e.g. common morbidities among siblings). In this population, the effects of within-household similarities were larger than within-village similarities (*21*). In our study, neither of these modifications affected the variance or the bias.

Although both diarrhoea and dysentery prevalence estimates showed substantial relative bias (diarrhoea, up to 17%; dysentery, up to 23%), the bias for estimates of diarrhoea was always positive, whereas the bias for estimates of dysentery varied. By increasing the sample size, variance decreased, and, to a lesser extent, so did bias for estimates of both diarrhoea and dysentery prevalence. This can be explained by the increasing proportion of the total population sampled. The largest sample size (1000) included approximately 1 out of every 4 children in the population. In some small wards, when the largest sample size was used, every child was selected.

Further studies would be needed to determine whether these results can be applied in other settings, or whether seasonal variations in disease prevalence would alter these findings. Modifications to EPI cluster sampling may provide more accurate estimates and should be examined more closely, perhaps using computer simulation techniques (*22*).

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Résumé

Efficience du sondage en grappes du PEV pour l'estimation de la prévalence de la diarrhée et de la dysenterie

La diarrhée et la dysenterie sont des causes majeures de morbidité et de mortalité chez l'enfant dans de nombreux pays en développement; le traitement et la prévention ne sont toutefois possibles que si l'on dispose en temps voulu d'informations exactes sur leur prévalence. Un système de surveillance utilisant des sondages en cours permet d'obtenir de telles données.

Plusieurs méthodes d'enquête par sondage sont utilisables, mais les ressources disponibles et le niveau d'exactitude requis sont des facteurs contraignants. Le sondage aléatoire simple, s'il est fait correctement, fournit des estimations précises et exactes (c'est-à-dire des estimations non biaisées, ayant une variance faible). Les conditions de réalisation du sondage aléatoire simple sont toutefois difficiles, voire impossibles, à remplir. Il est en effet particulièrement malaisé d'obtenir une base de sondage appropriée (c'est-à-dire une liste de tous les membres de la population); de plus, si la population est géographiquement dispersée, et que certains membres habitent des secteurs éloignés ou inaccessibles, les entretiens risquent de poser des problèmes logistiques supplémentaires.

Une méthode simplifiée de sondage en grappes mise au point par le Programme élargi de Vaccination (PEV) de l'OMS permet de résoudre ces difficultés, en utilisant à la fois une base de sondage en grappes, et une stratégie de sondage qui limite la dispersion géographique des sujets sélectionnés. Ces avantages sont toutefois obtenus au prix de la perte d'une certaine exactitude et d'une certaine précision des estimations obtenues. Cette étude se propose de mesurer comment l'économie de ressources compense l'augmentation du biais et de la variance des estimations.

On constate que la méthode de sondage en grappes du PEV présente systématiquement un biais relatif positif qui atteint 17% (par rapport à la moyenne pour la population) pour les estimations de prévalence de la diarrhée. Concernant la dysenterie, le biais est tantôt positif, tantôt négatif. Les estimations de prévalence de la diarrhée sont plus précises que celles obtenues avec le sondage

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en grappes classique (sondage aléatoire stratifié); les estimations de prévalence de la dysenterie sont tantôt plus précises, tantôt moins précises, que celles obtenues par sondage en grappes classique.

Le sondage en grappes du PEV a exigé en général des déplacements moins lointains (la distance sert d'indicateur de coût dans l'analyse) que le sondage en grappes classique; toutefois, comme on s'y attendait, cet avantage a disparu au fur et à mesure que des modifications destinées à garantir une plus grande dispersion géographique des sujets étaient apportées à la méthode du PEV.

Dans la mesure où, avec le sondage en grappes du PEV, l'on observe une variation du biais avec la maladie étudiée, il est possible que les différences de répartition spatiale des variables considérées (morbidité par exemple) modifient les estimations de prévalence lorsqu'on utilise cette méthode. Les estimations obtenues grâce à la méthode du PEV peuvent donc avoir une exactitude acceptable dans certaines situations. Provisoirement, ces estimations seront toutefois interprétées avec une certaine prudence. Reste que le biais étant constant, comme c'est le cas pour les estimations de prévalence de la diarrhée, la surveillance des tendances dans la population n'en est pas affectée.

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