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9. ABSTRACT

White wheat flour enriched with lysine was the only source of protein in the diet of six convalescent malnourished infants for 3-6 months. At 8% protein calories the equivalents of 0.1, 0.2, and 0.4% lysine enrichment were equally effective in supporting normal linear growth, weight gain, nitrogen retention, serum proteins, plasma amino acids, and liver morphology. Between 6.4 and 7.3% protein calories there was almost certainly an advantage to 0.2% over 0.1% enrichment, and possible to 0.4% over 0.2% enrichment, by the same parameters. If wheat is to be enriched with lysine in those areas where it is the main source of protein for many infants and small children, and where dilution by nonprotein calories is the rule, at least 0.2% enrichment is recommended.

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Lysine enrichment of wheat flour: prolonged feeding of infants[1,2]

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In a previous report (1), we outlined the results of short-term comparisons between casein, wheat flour alone, and the same enriched with three different levels of lysine, as the sole source of protein in the diet of convalescent malnourished infants. The studies were carried out with a high protein flour obtained by air classification of ordinary white wheat flour. At the first level of enrichment, *WL-1*, enough L-lysine-HCl was added to make lysine theoretically no more limiting than threonine; this was the equivalent of 0.12% enrichment of ordinary flour. At the second level, *WL-2*, enough lysine was added to make its content, as a fraction of essential amino acids, equal to that in human breast milk; this was the equivalent of 0.2% enrichment of ordinary flour. At the third level, *WL-3*, the lysine content of breast milk, as a fraction of total nitrogen, was approximated; this was the equivalent of 0.4% enrichment of ordinary flour.

All three enriched flours supported rates of weight gain and apparent nitrogen retentions that were significantly superior to those from unenriched flour. In theory, maximal enhancement of biological value should have been obtained with *WL-1*; in practice, the results with *WL-2* and *WL-3* were superior to those with *WL-1*, practically equaling the results with casein. We believe that the additional benefit apparent is the result of rapid absorption of the free lysine, with some wastage due to deamination. Unpublished determinations of postprandial plasma amino acids in children receiving the enriched flours reveal that lysine peaks at 1 hour, whereas the other essential amino acids generally do so at 3–4 hr.

The present report details our observations in six convalescent malnourished infants who received the enriched flour as the sole source of dietary protein for 3–6 months. Two of the infants received *WL-1*, two *WL-2*, and two *WL-3*. Because of the significantly poorer retentions of nitrogen from unenriched flour, prolonged feeding was not attempted.

Materials and methods

The subjects of these studies were originally admitted to the British American Hospital suffering from severe malnutrition, diarrhea, and other infections. They were given appropriate dietary and supportive therapy until their acute problems were brought under control, and then given a variety of diets, usually milk-based, until a steady rate of weight gain was established. Prolonged feeding of enriched flour was begun 1.5–9 months later (Fig. 1), when weight age had approximately equaled height age and serum albumin was at least 3.44 g/100 ml (Table 1). In four of the infants, *No. 202, 207, 212,* and *222,* serum albumin exceeded 4.0 g/100 ml.

Composition of the diets, as well as vitamin and mineral supplementation, were exactly as in the previous comparative studies (1). Immediately before and after the wheat diets an isocaloric, isonitrogenous casein diet was given for comparative nitrogen balance studies. Except for the days of these latter studies, which were carried out in a metabolic unit, the infants were in a convalescent

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unit where they enjoyed a good deal of freedom and stimulation. Consumption of diets was not as complete as in the metabolic unit and no attempt was made to measure spillage, vomitus, or unconsumed amounts.

Hematologic values were determined regularly and showed consistently satisfactory trends. Serum total proteins and albumin were also estimated regularly; only the albumin levels at the beginning, at the time of any change in the protein or calorie intakes, and at the end of the study, are considered in this report. Fasting plasma free amino acids were estimated at the end of the wheat periods and at other times during their consumption.

The duration of the periods in which enriched flour was the only source of dietary protein ranged from 92 to 182 days. In two of the infants the percentage of calories as protein remained constant; in the other four, changes were made as indicated in Table 1.

Results

The first infant, *No. 202*, received *WL-1* for 92 days, with only 6.6% of calories as protein, an extremely critical level. In the initial comparison with casein, apparent nitrogen retention during the first three days on wheat was quite low, but then increased steadily (Table 2). The markedly increased retention from casein in the terminal comparison, from 62 to 114 mg/kg per day, suggests that a state of mild protein deficiency existed at the end of the wheat period (2). The moderate but significant fall in serum albumin and the presence of 3+ (basis 0–4+) liver fat on biopsy at the end of the wheat diet are confirmatory. Nevertheless, there was a satisfactory parallel increase in height and weight ages (Table 1). Figure 1 indicates that the rapid "catch-up" growth to be expected in this child was already complete when the wheat diet was begun and that his height "quotient" (100 × height age/chronologic age) continued to increase at a slower rate. Horizontality of the height quotient can be interpreted in two ways, either the diet is not adequate to permit catch-up, or this is complete and the maximum quotient has been reached.

The second child, *212*, was much older than the others on admission and at the beginning of the *WL-1* diet. This was given at 8.0% protein calories for 42 days, at 7.9% for 40 days, and at 7.3% for the final 51 days,

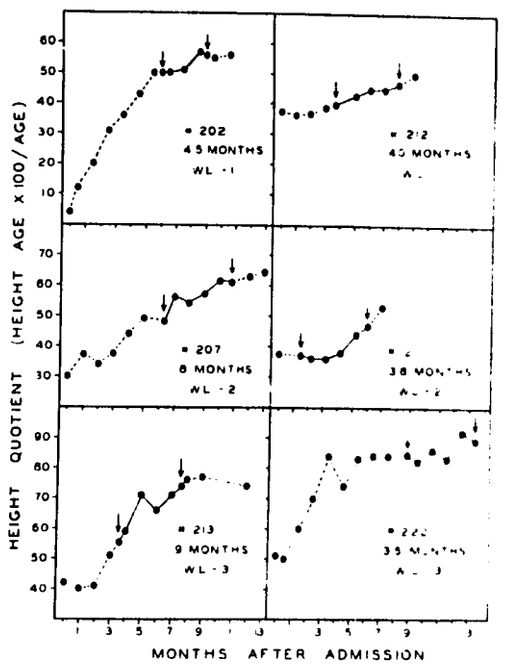

FIG. 1. Evolution of linear growth in six malnourished infants who received wheat flour enriched with lysine (*WL-1*, *WL-2*, and *WL-3*) as the sole source of dietary protein during at least three months of their convalescence. Height is expressed as the developmental quotient to demonstrate "catch-up". Solid line between two arrows is period of *WL* diet. Age in months at time of admission is given for each child.

for a total of 133 days. These levels are considerably less critical than was true of *202*, particularly when the more advanced age of *212* is taken into account. In the initial comparison, the apparent retention of N from *WL-1* actually exceeded that from casein (Table 2), probably as the result of better absorption, normal day-to-day variation, and the inherent errors of the method. In the terminal comparison the reverse was true, probably for the same reasons. Neither one of the differences was significant. Serum albumin remained at normal levels throughout, and a final liver biopsy was entirely normal by light microscopy, both suggesting adequacy of the protein intake. Gains in height age exceeded those in chronologic age, whereas those in weight age far exceeded both (Table 1). From Fig. 1 it is evident that *WL-1*, at the levels given, supported the already established steady gain in height quo-

TABLE 1

Evolution of height age, weight age, and serum albumin during prolonged feeding of wheat flour enriched with lysine (*WL*) at three different levels as the only source of dietary protein

Case number, sex	Initial ages[a] in months			Diet	% Protein calories	Number of days	Progress in months			Serum albumin, g/100 ml	
	CA	HA	WA				CA	HA	WA	Δ	Final
202M	10	5½	6½	WL-1	6.6	92	3	2	2	−0.43	3.85
212M	43	17	18	WL-1	8.0	42				−0.31	4.12
				WL-1	7.9	40				0.09	4.21
				WL-1	7.3	51	4½	5½	11½	0.15	4.36
207M	15	7½	7½	WL-2	8.0	38				0.06	4.49
				WL-2	7.3	81				−0.20	4.29
				WL-2	8.0	18	4½	4½	4½	0.01	4.30
211F	15	5½	4½	WL-2	6.7	46				−0.38	3.06
				WL-2	6.4	29				0.17	3.23
				WL-2	8.0	62	4½	3½	12½	0.62	3.85
213M	12	6½	6	WL-3	8.0	126	4	6½	10	0.73	4.31
222M	12	10	9	WL-3	6.6	7				−0.25	4.20
				WL-3	8.0	175	6	6½	6½	0.15	4.35

[a] Chronologic age (CA), height age (HA) and weight age (WA).

TABLE 2

Nitrogen balances during prolonged feeding of wheat flour enriched with the equivalent of 0.12% lysine (*WL-1*) as the only source of dietary protein

Case number, sex	Age, months	Diet						Nitrogen balances			
		Protein	g/kg per day	Lysine, mg/kg per day	kcal/kg per day	% Protein	Number of days	mg/kg per day		% of intake	
								Intake	Retention	Absorption	Retention
202M	10	C	1.65		100	6.6	6	264	66	87	25
		WL-1	1.65	17.7	100	6.6	3	264	36	84	14
							3	264	52	90	20
							3	264	61	86	23
	13	WL-1	1.65	17.7	100	6.6	12	259	62	82	24
		C	1.65		100	6.6	9	264	114	85	43
212M	43	C	2.0		100	8.0	6	320	82	87	26
		WL-1	2.0	21.5	100	8.0	6	320	104	95	32
	44	WL-1	2.0	21.5	100	8.0	6	319	78	80	24
	48	WL-1	2.0	21.5	110	7.3	6	320	95	82	30
		C	2.0		110	7.3	6	313	120	88	38

Comparisons with casein (C) at beginning and end of study.

tient, as rapid as can be expected at his age (3).

The third infant, *207*, received *WL-2* at 8.0% protein calories for 38 days, at 7.3% for 81 days, and again at 8.0% for a final 18 days, for a total of 137 days. In the initial comparison with casein, N retentions during the second and third 3-day *WL-2* periods were adversely affected by anorexia and vomiting, the result of an incipient infection. They were even more adversely affected, for a similar reason, during the *WL-2* period of

the final comparison with casein (Table 3). Despite these seemingly poor retentions, progress in height and weight ages exactly matched the advance in chronologic age. Serum albumin remained normal (Table 1). A final liver biopsy was also normal. From Fig. 1 it is also evident that accelerated catch-up linear growth was maintained during the *WL-2* feeding. All these observations are indicative of dietary protein adequacy.

The fourth infant, *211*, received *WL-2* at 6.7% protein calories for 46 days, at 6.4% for 29 days, and at 8.0% for 62 days, for a total of 137 days. She had been in the hospital for only 6 weeks and her serum albumin was 3.44 g/100 ml at the start of the *WL-2* diet. During the initial comparative balances (Table 3), N retention from *WL-2* was considerably lower than from casein, probably because of the critical level of protein intake. During the final comparison, at a more favorable level of protein calories, N retention from *WL-2* was considerably improved, but was still followed by increased retention from casein, suggestive of previous dietary protein inadequacy. Serum albumin (Table 1) fell initially but then stabilized and recovered. Gain in height age was slightly less than that in chronologic age; gain in weight age was much greater. During the first 3.5 months in the hospital, the last two on *WL-2*, there was no progress in height quotient (Fig. 1), but during the following 2.5 months on *WL-2* there was rapid catch-up. It is not unusual to see an original lag in linear growth catch-up, but it may well be that dietary protein was adequate only when it provided 8.0% of total calories. Final liver biopsy was normal.

The fifth infant, *213*, received *WL-3* at 8.0% protein calories during 126 days. Initial and final retentions of N were at least equal to those from casein (Table 4), serum albumin increased to high normal levels, and gains in height and weight ages surpassed those in chronologic age (Table 1). Accelerated catch-up growth was maintained (Fig. 1), and final liver biopsy was normal.

After an initial week at 6.6%, *222* received *WL-3* at 8.0% protein calories for 175 days. Initial and final retentions of N were very similar to those from casein (Table 4), serum albumin was maintained at normal levels, and progress in height and weight ages was slightly more than that in chronologic age (Table 1), so that a modest gain in height quotient was apparent (Fig. 1), even though maximal catch-up had apparently been achieved already. Final liver biopsy was normal.

The results of the fasting plasma free

TABLE 3

Nitrogen balances during prolonged feeding of wheat flour enriched with the equivalent of 0.2% lysine (*WL-2*) as the only source of dietary protein

Case number, sex	Age, months	Diet						Nitrogen balances			
		Protein	g/kg per day	Lysine, mg/kg per day	kcal/kg per day	% Protein	Number of days	mg/kg per day		% of intake	
								Intake	Retention	Absorption	Retention
207M	15	C	2.0		100	8.0	6	330	113	94	35
		WL-2	2.0	35.9	100	8.0	3	318	98	98	31
							3	288	28	87	10[a]
							3	313	58	90	19[a]
	19	WL-2	2.0	35.9	100	8.0	6	188	26	87	14[a]
		C	2.0		100	8.0	9	270	85	91	31[a]
211F	15	C	2.5		150	6.7	9	400	138		34
		WL-2	2.5	44.8	150	6.7	6	376	56		15[a]
							6	400	41		10
	19	WL-2	2.0	35.9	100	8.0	9	320	98		31
		C	2.0		100	8.0	6	320	149		47

Comparisons with casein (C) at beginning and end of study. [a] Incomplete intake due to anorexia or vomiting.

TABLE 4

Nitrogen balances during prolonged feeding of wheat flour enriched with the equivalent of 0.4% lysine (*WL-3*) as the only source of dietary protein

Case number, sex	Age, months	Diet						Nitrogen balances			
								mg/kg per day		% of intake	
		Protein	g/kg per day	Lysine, mg/kg per day	kcal/kg per day	% Protein	Number of days	Intake	Retention	Absorption	Retention
213M	12	C	2.5		125	8.0	6	400	143	91	36
		WL-3	2.5	90.8	125	8.0	9	400	144	88	36
	16	WL-3	2.5	90.8	125	8.0	6	381	149	89	39
		C	2.5		125	8.0	3	293	54	86	18[a]
							6	378	145	92	38
222M	12	C	1.65		100	6.6	6	264	73	85	28
		WL-3	1.65	63.9	100	6.6	3	264	107	85	40
		WL-3	2.0	77.4	100	8.0	6	320	90	88	28
	17	WL-3	2.0	77.4	100	8.0	12	320	90	86	28
	18	WL-3	2.0	77.4	100	8.0	3	286	77	80	27[a]
		C	2.0		100	8.0	6	314	111	91	35

Comparisons with casein (C) at beginning and end of study. [a] Incomplete intake due to anorexia or vomiting.

amino acid determinations in the six children during and at the termination of prolonged feeding are given in Table 5, compared with the mean values for the three levels of enrichment in our previous study (1). If the ratio of essential to total amino acids is taken as a reasonable estimate of the adequacy of protein in the diet (4, 5), it is apparent that the diets were most adequate for patients *No. 213* and *222*, both receiving *WL-3*, for *207* on *WL-2*, and *212* on *WL-1*. In *No. 213*, *222*, and *212*, this is confirmed by the nitrogen balances, serum albumins, liver biopsies, and rates of growth. For *207*, on *WL-2*, only the nitrogen balances were unsatisfactory (Table 3), but as indicated, this was probably a transitory phenomenon. The poorer essential amino acid–total amino acid (EAA/AA) ratios of *202*, on *WL-1*, were probably due to the more critical level of intake, and those of *211*, on *WL-2*, were probably due to the much earlier stage of convalescence and the initially more critical level of intake. There was generally an inverse relation between plasma lysine and threonine levels, as in our previous studies.

Discussion

Previous comparative studies documented the fact that the equivalent of 0.1% lysine enrichment of wheat flour significantly increased its protein value, and suggested that enrichment to the 0.2 or 0.4% level might result in further benefit (1).

In the present study we have attempted, by prolonged feeding, to determine if there is indeed further benefit to be expected from 0.2 and 0.4% enrichment. It is quite apparent that the most highly enriched flour, identified as *WL-3*, when fed at 8% protein calories, was able to support "normal" growth for many months. All the indicators of adequacy used: linear growth, weight gain, apparent nitrogen retention, serum albumin, plasma free amino acids, and liver biopsy, were consistently satisfactory.

Interpretation of the results of the nitrogen balances on *WL-2* was clouded by periods of incomplete consumption of the diet or vomiting, probably the result of inapparent infection. Despite this, the results in one case, at 7.3 to 8.0% protein calories, were satisfactory by all other criteria. In the other child receiving *WL-2*, the diet, at 6.4–6.7% calories for 75 days, was begun very early in recovery, when serum albumin was still low, and the results were only marginally satisfactory. After protein was increased to 8% of calories, the results were generally very satisfactory.

The oldest child in the study, *212*, who

TABLE 5

Fasting plasma free amino acids during prolonged feeding of *WL* diets

Case number and diet	Protein, % kcal	Diet, days	AA μmoles/ml	EAA μmoles/ml	EAA-AA	Lys-EAA	Thr-EAA
202-WL-1	6.6	9	1.744	0.381	0.215	0.139	0.136
202-WL-1	6.6	31	1.826	0.331	0.181	0.139	0.112
202-WL-1	6.6	92	1.404	0.340	0.242	0.271	0.071
212-WL-1	8.0	9	2.187	0.669	0.306	0.164	0.221
212-WL-1	7.3	137	2.825	0.919	0.325	0.159	0.198
WL-1[a]	6.4-8.0	15+	2.211	0.537	0.242	0.166	0.167
207-WL-2	8.0	9	2.028	0.501	0.247	0.146	0.110
207-WL-2	8.0	137	2.334	0.591	0.253	0.134	0.186
211-WL-2	6.7	8	1.950	0.342	0.175	0.342	0.047
211-WL-2	8.0	88	2.227	0.485	0.218	0.151	0.109
211-WL-2	8.0	103	2.630	0.527	0.200	0.118	0.116
211-WL-2	8.0	137	2.277	0.519	0.228	0.229	0.173
WL-2[a]	6.4-8.0	15+	2.137	0.515	0.240	0.178	0.142
213-WL-3	8.0	9	2.575	0.619	0.240	0.221	0.132
213-WL-3	8.0	31	2.574	0.752	0.292	0.174	0.152
213-WL-3	8.0	126	2.744	0.857	0.312	0.145	0.209
222-WL-3	8.0	18	1.801	0.561	0.311	0.196	0.223
222-WL-3	8.0	161	2.032	0.521	0.256	0.177	0.190
WL-3[a]	6.4-8.0	15+	2.335	0.576	0.237	0.191	0.127

Total (AA) and essential (EAA) amino acids, essentials as a fraction of totals (EAA/AA), lysine (Lys/EAA) and threonine (Thr/EAA) as the molar fraction of essentials, compared with the mean values after short-term feeding (1). [a] Mean values for each of the three levels of enrichment in the previously reported short-term comparative studies (1).

might have been expected to have the lowest protein requirement, received 7.3–8.0% of calories as protein from *WL-1*, the lowest level of enrichment. Results were as satisfactory as those of any other child in the study. The only clearly unsatisfactory results, by all criteria, were those in *202*, who received only 6.6% of calories as protein from *WL-1*. The only child with which he can be compared fairly is *211* (during the first 75 days), and the results were probably inferior.

The nature of these studies, with no proper controls, makes it difficult to draw conclusions. It does seem apparent, however, that for infants and children beyond 6 months of age, wheat flour enriched with 0.1–0.4% lysine, when it provides at least 8% of calories as protein, can serve as the only source of protein in the diet for many months, supporting growth which is adequate by all our criteria. If enriched flour provides between 6.4 and 8.0% of calories, 0.2 and 0.4% enrichment probably provide a higher margin of safety than 0.1%. If wheat flour is to be enriched with lysine in those areas where it is the main source of dietary protein for a significant number of infants and preschool children, we would recommend at least 0.2% enrichment.

Summary

White wheat flour enriched with lysine was the only source of protein in the diet of six convalescent malnourished infants for 3–6 months. At 8% protein calories the equivalents of 0.1, 0.2, and 0.4% lysine enrichment were equally effective in supporting normal linear growth, weight gain, nitrogen retention, serum proteins, plasma amino acids, and liver morphology. Between 6.4 and 7.3% protein calories there was almost certainly an advantage to 0.2% over 0.1% enrichment, and possibly to 0.4 over 0.2% enrichment, by the same parameters. If wheat is to be enriched with lysine in those areas where it is the main source of protein for many infants and small children, and where dilution by nonprotein calories is the rule, at least 0.2% enrichment is recommended.

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