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Effect of Amino Acid Composition of Cereal-Based Diets on Growth of Preschool Children[1]

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Cereals form the major source of protein and calories in the diets of people in developing countries. Surveys conducted in South India (1, 2) have shown that cereals supply 70–80% of the dietary protein of preschool children.

In our laboratories, the efficacy of protein supplements has been tested by assessing their effect on the growth of preschool children over periods of 6–8 months. Over the last 2 years, children had been fed diets based on two different cereals, wheat and rice, and had been found to grow at different rates, although both diets provided the same quantity of protein and calories.

This paper describes the amino acid composition of the two diets and their effect on the rate of growth of preschool children.

MATERIALS AND METHODS

Subjects

The children who took part in the feeding trials were 2–5 years of age and in apparent good health, as determined by clinical examination. They were residents in an orphanage where the dietary intake was restricted to the foods described below.

Twenty-two children, 9 boys and 13 girls, were the subjects given the wheat-based diet. Twenty-four children, 12 boys and 12 girls, were given the rice-based diet.

Thirteen subjects (6 girls and 7 boys) were selected from the children who had been given the wheat-based diet and matched for age, sex, height, and weight with subjects on the rice-based diet. The heights and weights of the twenty-six children at the start of the trials were comparable to heights and weights of Indian rural children of the same ages (3).

Heights and weights were recorded at 4-week and 2-week intervals, respectively, by the same team of examiners. Minor illnesses were also recorded during the 6-month period of the trials.

In this paper, the rates of growth of the 13 children given the wheat-based diet are compared with the rates of growth of matched subjects given the rice-based diet.

Diets

Both diets provided 100 kcal and 2 g vegetable protein/kg body wt per day and were adequate in protein and calorie content (4, 5). Animal foods were completely excluded from the menu.

The diet based on wheat consisted of dishes prepared from wheat, pulses, vegetables, peanut and hydrogenated vegetable oils, and jaggery (crude sugar). The one based on rice contained pulses, vegetables, peanuts, hydrogenated vegetable oil, peanut oil, and jaggery in addition to cereal.

Four meals were served during the day. Each child's portion was weighed out at every meal and plate waste, if any, was recorded.

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Biochemical Methods

Nitrogen balance studies. Three-day nitrogen balance studies were carried out on 10 children on each diet.

Samples of the dishes, in the proportion eaten by each child, were weighed out daily and homogenized. Aliquots were taken for the estimation of protein, by the micro-Kjeldahl method.

Urine and feces were collected for 3 days. The 24-hr composites of stool were homogenized and analyzed for nitrogen. The nitrogen content of 24-hr urine collections was also analyzed.

Amino acid analyses. The 24-hr food samples representative of the average intakes of each group of children were collected twice a week throughout the feeding trials and refrigerated until transported to the laboratory where they were homogenized.

Five-gram aliquots of the homogenized food samples were weighed and transferred into glass ampules of 100 ml capacity. To these were added 30 ml 3 N HCl and the ampules were sealed. The mixtures were hydrolyzed at 120 C for 16 hr. The contents were cooled and filtered and the filtrate and washings diluted to 100 ml with distilled water. Five-milliliter aliquots were taken into beakers and evaporated again. This procedure was repeated three times. Finally, the amino acids were taken up in a citrate buffer, pH 2.2, filtered through a medium-porosity sintered glass funnel under vacuum and transferred to a 100-ml volumetric flask. For the estimation of the amino acids, 0.5-ml aliquots of the hydrolysate were employed, using the automatic amino acid analyzer. The amino acids of the 24-hr food samples were measured and the intake of the amino acids per child per day was calculated.

TABLE I

The age distribution of the children

Age, years	Number of children given the	
	Wheat-based diet	Rice-based diet
2	1	1
3	7	7
4–5	5	5
Total	13	13

TABLE II

Composition of the two diets and the average daily intake per child

Foodstuffs	Wheat-based diet, g	Rice-based diet, g
Wheat	189	20
Rice, parboiled		160
Blackgram dhal (*Phaseolus mungo*)	8	15
Potato	25	10
Peanut and hydrogenated vegetable oils	39	24
Jaggery (crude sugar)	19	25
Greens or vegetables	13	7
Peanuts		15

At the end of the feeding trials and after an overnight fast, venous blood was drawn into heparinized tubes from 6 of the 22 children on the wheat-based diet, and from 7 of the 24 on the rice-based diet for the estimation of plasma amino acids. The plasma was separated by centrifugation and frozen until the analysis.

The protein-free filtrate, obtained by the ultrafiltration of the plasma, was processed according to Benson and Patterson (6). Suitable aliquots (usually 0.5–1.0 ml) of the processed plasma were analyzed by a two-column automatic amino acid analyzer employing the buffer system developed for physiological fluids using Beckman spherical resins PA 28 and PA 35.

RESULTS

The number and the distribution of ages of the children who took part in the studies are given in Table I.

The composition of the diets based on wheat and rice is given in Table II and the nutritive values calculated from Indian food tables (7) are presented in Table III. Both diets provided 100 kcal and 2 g vegetable protein/kg body wt per day. There were minor variations with regard to mineral and vitamin content. The intakes of vitamin A, riboflavin, and ascorbic acid were low, compared with recommended allowances (8, 9). Apart from

TABLE III

Average daily intake of nutrients per child

Nutrients	Wheat-based diet	Rice-based diet
Calories	1,117	1,186
Protein, g	26.3	22.6
Fat, g	42.4	31.8
Calcium, mg	222	144
Iron, mg	17.6	14.2
Vitamin A, IU	757	848
Riboflavin, mg	0.3	0.4
Ascorbic acid, mg	12.5	7.3

TABLE IV

Essential amino acids and cystine supplied by the cooked foods (g/child per day)

Amino acids	Wheat-based diet	Rice-based diet
Threonine	0.49	0.66
Valine	0.70	0.81
Methionine	0.09	0.24
Isoleucine	0.47	0.54
Leucine	0.98	1.23
Phenylalanine	0.38	0.40
Lysine	0.54	0.88
Histidine		0.56
Cystine[a]	0.53	0.31
Arginine	0.70	1.44
Tyrosine	0.20	0.26
Tryptophan[a]	0.21	0.31

[a] Calculated from food tables (7).

occasional angular stomatitis, clinical deficiencies were not encountered during the trials.

The essential amino acids and cystine supplied by the cooked foods of the two diets are tabulated (Table IV). In Table V, the amino acids expressed as milligrams per kilogram body weight provided by the two diets are compared with the requirements of infants (10) and of children aged 10–12 years (11–13), as the amino acid requirements of preschool children are not established. When compared with the requirements of older children, the two diets supplied adequate amounts of leucine, isoleucine, phenylalanine, and valine; both diets were low in methionine content, the wheat-based providing less of this essential amino acid than the rice-based diet. The wheat-based diet was, in addition, deficient in lysine and threonine.

In Table VI, the amino acid content of the two diets is compared with the FAO reference amino acid pattern (5). Using

TABLE V

Comparison of the intake of amino acids of children on the two diets, with the amino acid requirements of infants and children

Amino acids	Amino acids, mg/kg body wt		Requirements	
	Wheat-based diet	Rice-based diet	Infants[a]	Children[b]
Isoleucine	37	45	126	30
Leucine	77	89	150	45
Lysine	44	72	103	60
Methionine	7	20	45	27
Phenylalanine	50	32	90	27
Threonine	28	55	87	35
Valine	55	67	105	33
Tryptophan	17[c]	27[c]	22	9
Arginine	55	119		

[a] See reference (10). [b] See references (11–13).
[c] Calculated from food tables (7).

TABLE VI

Amino acid content of the diets compared with the FAO reference amino acid pattern

Amino acid	Amino acid composition, %		
	FAO 1957	Wheat-based diet	Rice-based diet
Isoleucine	4.2	1.8	4.6
Leucine	4.8	3.7	7.6
Lysine	4.2	2.1	6.1
Phenylalanine	2.8	1.4	1.7
Tyrosine	2.8	0.9	1.2
Cystine	2.0	2.0[a]	1.3[a]
Methionine	2.2	0.3	1.1
Threonine	2.8	1.3	2.9
Tryptophan	1.4	0.8[a]	1.4[a]
Valine	4.2	2.7	3.6

[a] Calculated from food tables (7).

TABLE VII

Height, weight, and nitrogen balance data of children on the two diets

Subjects	Height, cm		Weight, kg		Nitrogen balance[a]			
	At start of study ± 1 SD	Increase ± 1 SD	At start of study ± 1 SD	Increase ± 1 SD	N intake	Urinary N	Fecal N	Average daily N balance
13 Children on rice-based diet	89.3 ± 5.0	4.04 ± 1.08	11.56 ± 1.10	0.49 ± 0.81	4.42 ± 0.43	1.49 ± 0.20	1.79 ± 0.25	+1.20 ± 0.41
13 Children on wheat-based diet	90.9 ± 6.3	2.62 ± 0.99	12.09 ± 1.64	0.58 ± 0.84	4.07 ± 0.15	1.71 ± 0.25	1.37 ± 0.33	+0.98 ± 0.22

[a] Average ± 1 SD of 10 children on each diet.

TABLE VIII

Plasma amino acids of children on the two diets

Amino acids	Children on wheat-based diet	Children on rice-based diet	American children of the same age[a]
Valine	1.52 ± 0.09	1.42 ± 0.66	2.88 ± 0.71
Proline	2.17 ± 0.28	2.59 ± 1.05	2.13 ± 1.06
Alanine	3.02 ± 0.47	4.19 ± 1.02	1.89 ± 0.53
Lysine	0.95 ± 0.18	1.15 ± 0.41	1.67 ± 0.52
Leucine	0.72 ± 0.05	1.01 ± 0.35	1.53 ± 0.49
Tyrosine	0.62 ± 0.09	0.81 ± 0.20	1.24 ± 0.53
Threonine	0.54 ± 0.10	0.79 ± 0.49	1.13 ± 0.39
Serine	1.25 ± 0.67	1.75 ± 0.86	1.07 ± 0.27
Glycine	1.46 ± 0.14	1.95 ± 0.36	1.07 ± 0.27
Histidine	1.26 ± 0.22	0.89 ± 0.20	0.91 ± 0.35
Taurine	0.90 ± 0.37	0.75 ± 0.47	0.91 ± 0.60
Isoleucine	0.45 ± 0.16	0.66 ± 0.21	0.90 ± 0.40
Tryptophan			0.82 ± 0.13
Arginine	0.91 ± 0.15	1.04 ± 0.21	0.77 ± 0.28
Phenylalanine	0.58 ± 0.04	0.59 ± 0.14	0.76 ± 0.26
Ornithine	0.50 ± 0.15	0.48 ± 0.14	0.67 ± 0.41
Cystine	0.61 ± 0.22	1.23 ± 1.17	0.25 ± 0.05
Methionine	0.29 ± 0.03	0.31 ± 0.09	0.25 ± 0.15
Butyric acid	0.07 ± 0.01	0.07 ± 0.03	0.22 ± 0.11
Aspartic acid	0.18 ± 0.01	0.06 ± 0.81	0.04 ± 0.02
Glutamic acid	3.10 ± 1.43	3.45 ± 2.16	[b]

Values (± 1 SD given below each value) are expressed as milligrams per 100 ml plasma. The values are an average of seven children on the wheat-based diet and six on the rice-based diet. [a] Average of eight analyses (15). [b] Not reported.

this parameter, the wheat-based diet was deficient in all the essential amino acids, whereas the rice-based diet was limiting in methionine, phenylalanine, and marginally so in valine.

The heights and weights of the children at the beginning and the increases at the end of the feeding trials are given in Table VII, together with the data on the nitrogen balances carried out on 10 children on each diet. The increases in height of the children on the rice-based diet were significantly greater than the increases in height of the children on the wheat-based diet ($P < 0.01$) and were comparable to the rate of growth of North American children in the 50th percentile (14). The differences in gain in weight and in nitrogen retention between the two groups of children were not of statistical significance.

Data on the plasma amino acids of seven children on the wheat-based diet and six children on the rice-based diet are given in Table VIII. The mean plasma amino acid levels of eight North American children of comparable age are also included for comparison (15).

The differences in the plasma levels of most of the essential amino acids between the children on the rice-based diet and the children given the wheat-based diet were not of statistical significance (*t* test). The plasma levels of histidine and of the nonessential amino acid, glycine, of the children on the two diets were of statistically significant difference at the 5% level.

When compared with the plasma amino acid levels of American preschool children, the plasma levels of essential amino acids, except valine, of the children on the rice-based diet showed no statistical difference; however, the differences in plasma level of valine between the two groups was significant at the 1% level.

The plasma amino acid levels of the children on the wheat-based diet were significantly lower than those of the American children; the differences in plasma levels of leucine, lysine, valine, and threonine were significant at the 1% level and those of isoleucine at the 5% level.

The plasma amino acid levels of methionine of both groups of children showed no statistical difference when compared with the values of the American children. Despite the difference in dietary intake of methionine of the children on the rice- and wheat-based diets, the plasma values did not show a significant difference.

DISCUSSION

In estimating the amino acid requirements of adults and children, the criterion of adequacy has been a positive nitrogen balance (11, 16) or "a zone of nitrogen equilibrium, where the difference between intake and excretion is within 5 percent" (17).

Holt and Snyderman (18), estimating the amino acid requirements of infants, found that satisfactory weight gain was a sensitive index of dietary amino acid adequacy.

Holt and his colleagues (18) have observed that "in the growing child, nitrogen retention continues, though at a lesser rate, even on deficient diets." Both groups of preschool children who took part in the present studies were in positive nitrogen balance, though their rates of growth were significantly different. Increases in weight often reflect increases in fat or water retention and not necessarily an increase of muscle mass (19). Satisfactory increases in height in the preschool children were, therefore, taken as evidence of an adequate supply of dietary amino acids.

Investigators have recorded the existence of a variation in the individual requirements of each essential amino acid. Swendseid et al. (20) have shown that, in some cases, the individual variation in requirements had been wide. In most instances, the highest value has been chosen by the investigators as the requirement for that particular age group.

The studies on amino acid requirements reported to date concern the requirements of young adults (17, 20, 21), schoolchildren (11–13), and infants (10, 18). The amino acid requirements of preschool children have not been reported. Preschool children are growing more rapidly than older children but at a slower rate compared with infants. Therefore, one may assume that their requirements are greater than those of schoolchildren but less than those of infants.

Snyderman et al. (22) have commented on the lowered stool nitrogen and lowered serum amino acid levels of infants fed mixtures of amino acids in contrast to infants on naturally occurring protein, which they speculate may be due to more rapid absorption and utilization of the added amino acids rather than those in dietary proteins. In the present study, the amino acids were provided only in the form of dietary protein and, therefore, represent a normal situation.

One method of assessing the quality of a dietary protein is to compare the amino acid content with that of a protein of known quality, such as milk or egg, or to that of the FAO reference protein (5). The wheat-based diet provided less of the essential amino acids than the FAO amino acid reference pattern, and the rice-based diet was limiting in phenylalanine and methionine. Since the formulation of the FAO reference protein in 1957, there has been evidence to show that the levels of methionine recommended are higher than human requirements. Decreased nitrogen retention was observed in children fed corn-masa supplemented with methionine at the recommended levels as shown by Scrimshaw et al. (23) and Bressani et al. (24). Nitrogen retention increased when the methionine intake was reduced.

However, when the amino acids provided by the two diets are expressed as milligrams per kilogram body weight and are compared with the requirements of children, the wheat-based diet was found to provide inadequate amounts of isoleucine, methionine, lysine, threonine, and phenylalanine. The rice-based diet was low in methionine and phenylalanine content.

Phenylalanine

Leverton et al. (25) have shown that phenylalanine requirements are decreased in the presence of adequate amounts of tyrosine. Young women maintained positive nitrogen balance on intakes of 420 mg phenylalanine without tyrosine and were also found to be in positive balance on an intake of phenylalanine as low as 220 mg/day when the diet contained 900 mg tyrosine.

The phenylalanine content of the rice diet used in the present study appears low in comparison with the FAO reference protein pattern. The actual amounts of phenylalanine supplied by the wheat- and rice-based diets were 380 and 395 mg/day, and the tyrosine content was 201 mg and 255 mg/day, respectively. Under the circumstances, it is unlikely that phenylalanine was limiting in either diet.

Threonine

The requirements of threonine have been established at 87 mg/kg for infants (10) and at 33 mg/kg for schoolchildren (13). The rice-based diet provided 55 mg/kg. The wheat-based diet supplied 28 mg/kg, a level less than the requirements of schoolchildren. Threonine may have been one of the limiting amino acids in the wheat-based diet

Isoleucine

Swendseid and Dunn (21) have shown that young women maintained positive nitrogen balance on diets providing 250–450 mg isoleucine/day. Infants require 126 mg isoleucine/kg body wt as described by Holt et al. (18). The requirement of 11-year-old children was 30 mg/kg per day as calculated by Nakagawa et al. (11). The preschool children in the present study probably required much less isoleucine than infants though more than the 11-year-olds described by Nakagawa and his colleagues. The wheat-based diet provided only a little more isoleucine than the 37 mg/kg recommended for older children; however, on the rice-based diet the children obtained 45 mg/kg per day. The isoleucine content of the wheat-based diet was in all probability one of the limiting factors of the diet. The growth of the children on the rice-based diet would support the argument that the isoleucine content was adequate at 45 mg/kg.

Lysine

With regard to lysine, the diet based on wheat provided 540 mg/child per day or

44 mg/kg. This intake of lysine is lower than that stated to be required by schoolchildren reported by Nakagawa et al. (12).

Supplementation of the wheat-based diet with L-lysine monohydrochloride to provide a total of 730 mg/child per day or 60 mg/kg per day resulted in an improved rate of growth in a group of children of the same age reported earlier by Pereira et al. (26). The children on the lysine-supplemented wheat grew at the rate of 0.54 cm/month, compared with the rate of growth of the children on the wheat-based diet, who grew, on an average, 0.43 cm/month. The children on the rice-based diet that provided 72 mg lysine/kg increased in height at an average rate of 0.67 cm/month, a rate comparable to the 50th percentile of North American children. It is apparent, from the increased rate of growth of children on lysine-supplemented wheat, that lysine was a limiting amino acid in the wheat-based diet.

Methionine

Methionine requirements are related to, and vary with, the dietary content of the other sulfur-containing amino acids, cystine and cysteine. On diets that provided 200 mg cystine and 350–550 mg methionine/day, Swendseid et al. (20) found that young women were in positive nitrogen balance. Holt et al. (10) have shown that infants require 45 mg/kg daily, and in 10- to 11-year-old children, 27 mg/kg methionine without cystine was found to be adequate (12).

In the present study, the wheat- and rice-based diets provided 270 and 190 mg cystine/day, respectively, according to calculations from food tables. The methionine content of the wheat-based diet was low, providing 7 mg/kg; that of the rice-based diet was also low, although to a lesser extent than the wheat-based diet, and provided 20 mg/kg. The quantities of methionine and cystine provided by the rice-based diet were sufficient to maintain very satisfactory rates of growth. The methionine and cystine contents of the wheat-based diet may have been limiting. It is of interest that, despite the poor dietary methionine content, the levels of methionine in the plasma of children on the wheat-based diet were not dissimilar to those of normal American children of comparable age (15). The nutritional relationship between dietary amino acid content and amino acid levels in the plasma of fasting subjects is not well understood. The plasma levels of methionine of the children on the wheat-based diet are comparable to the levels of American children and probably indicate an adequate intake. The deficiencies of the other amino acids, isoleucine and lysine, apparently contributed in greater part than methionine to the less satisfactory growth of children on the wheat-based diet.

In conclusion, the methionine content of the rice-based diet is seemingly inadequate in comparison with the FAO provisional pattern. In this study, however, the amino acid pattern of the rice-based diet sustained a rate of growth in preschool children comparable to that of North American children of the same age. Our findings are in accordance with those of Bressani et al. (24) and Scrimshaw et al. (23) and suggest that the recommended level of intake of methionine in the FAO amino acid pattern is higher than the actual requirement.

SUMMARY

The efficacy of two diets, one based on wheat and the other on rice, on the growth (height) of preschool children has been tested in trials lasting 6 months. Both diets provided 2 g vegetable protein and 100 kcal/kg body wt. The increase in height of the children fed the rice-based diet was 0.67 cm/month and that of the children on the wheat-based diet,

0.43 cm/month. The children maintained positive nitrogen balance on both diets.

Amino acid analyses of the cooked foods showed the wheat-based diet to be limiting in lysine, methionine, threonine, and isoleucine and the rice-based diet in methionine. However, the rate of growth of the children fed the rice-based diet suggests that methionine was not a limiting factor; the recommended intake of methionine (FAO/WHO) is likely to be higher than the requirements of preschool children.

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