APPENDIX V-h

PN AAS-563

ISN 39

Plasma Free Amino Acids of Infants and Children Consuming Wheat-Based Diets, With and Without Supplemental Casein or Lysine

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SUBJECT:

Progress Report - January 12, 1981
Research Contract AID/ta-C-1286
Clinical Assays of High Protein Foods

ABSTRACT

Fasting and postprandial plasma free amino acids (AA) were measured in infants and small children consuming five energy-sufficient wheat-based diets: (a) 44 to 50% of calories (Kcal) and all of 6.6% protein (pro) Kcal from wheat, (b) the same plus enough casein to make 8.0% pro Kcal, (c) 75% Kcal and all of 9.8% pro Kcal from wheat, (d) the same with 0.2% lysine added to the wheat, and (e) identical to diet c, but following diets c and d.

On the 9th day of diet a, clearly inadequate in available protein because of its severe lysine deficiency, 16 subjects demonstrated low fasting TEAA/TAA (total essential AA/total AA molar concentrations) ratios, with significant (sig.) declines 3 ($P<0.05$) and 4 hours ($P<0.01$) postprandially (p.c.). Fasting molar fractions of each essential AA (EAA/TEAA) were not different from those of subjects consuming isonitrogenous milk protein diets, with the exception of slightly lower Lys/TEAA and higher Thr/TEAA. Postprandially there were sig. ($P<0.001$) declines in Lys/TEAA and increases in Ile, Tyr and Phe/TEAA, only a modest ($P<0.05$) decline in Thr/TEAA. After 3+ months of diet b, 4 subjects had higher ($P<0.05$) fasting Lys/TEAA with marked ($P<0.01$) p.c. declines, much lower ($P<0.001$) fasting Thr/TEAA, and still sig. ($P<0.05$ or <0.01) p.c. increases in Ile, Tyr and Phe/TEAA.

After 28 to 89 days of diet c in 10 subjects there was a sig. ($P<0.001$) p.c. increase in TEAA/TAA; fasting Lys/TEAA was lower than after diet a and its sig. ($P<0.01$ and <0.001) p.c. decline reached lower levels; fasting Thr/TEAA was again elevated, with a modest ($P<0.05$) decline at 3 hours; there were sig. ($P<0.01$ or <0.001) p.c. increases in Ile, Tyr and Phe/TEAA. When lysine was added in 7 of the 10 subjects

(diet d), after 8 to 50 days fasting Lys/TEAA was sig. ($P<0.05$) higher, with no p.c. decline; fasting Thr/TEAA was lower ($P<0.02$); the only sig. p.c. change was an increase ($P<0.01$) in Phe/TEAA. After 15 to 29 days of withdrawing the added lysine in 5 of the 7 subjects (diet e), the plasma AA changes were very similar to those after the identical diet c; fasting Lys/TEAA was lower, however, suggesting a greater depletion of body lysine pools.

Fasting plasma free AA tend to reflect the diet TEAA/TAA ratio, protein/calorie ratios, protein "status", and the first-limiting EAA in diet (after prolonged consumption). The second-limiting EAA may actually be elevated. Postprandial EAA/TEAA declines clearly identify the first-limiting EAA; increases may be due to relative diet excess or delays in clearing from plasma.

Key Words

Plasma Amino Acids
Infants and Children
Wheat
Lysine

Earlier observations on infants and small children consuming proteins with methionine or lysine as their first-limiting factor have suggested that plasma free amino acid (AA) levels could be used to identify limiting AA in the diet. Although fasting methionine level was relatively resistant to depression by consumption of proteins limiting in methionine, a statistically significant fall in its molar concentration and especially in its molar fraction of total essential amino acids (Met/TEAA) was demonstrated three and four hours postprandially (1). After nine days of consumption of a wheat-based diet neither the molar concentration of lysine nor the Lys/TEAA fraction was depressed in fasting plasma, but after 18 and 27 days there was a significant fall in both (2). Three and four hours after the consumption of a single meal of the same diet (6.4% wheat protein calories) there was a significant fall in plasma lysine and Lys/TEAA. When a single meal provided 8.0% wheat protein calories plus 4.0% additional protein calories from casein, in three of four children there was no postprandial plasma lysine depression, even though lysine was still presumed to be the essential amino acid in shortest supply (2). Although postprandial plasma AA changes of infants consuming exclusively rice protein were muted, probably because of poor nitrogen (N) absorption from this source, there was still a significant fall in Lys/TEAA ratios, lysine being the recognized first-limiting amino acid (3).

Additional studies carried out with wheat diets have made possible further observations on the role of plasma AA determinations in deciding whether lysine deficiency was limiting growth in the subjects under observation. In one study infants and children who received 44 to 50% of total calories and all of 6.6% protein calories from 82% extraction

wheat flour were shown to retain on the average only 50% of the N retained from isonitrogenous casein diets (4). Fasting and postprandial plasma samples were obtained on the ninth day of consumption of the wheat diets. In another study infants and children received 50% of calories as the same wheat flour, with enough casein added to bring protein calories from 6.6 up to 8.0%. During three months they maintained accelerated ponderal and linear growth without evidence of protein deficiency. In the same study another group of children received for a similar length of time diets in which wheat provided 75% of calories and 100% of protein. In most of this group lysine was added to the wheat during part of the study, usually the second of three months, and produced significant increases in both N retention and rate of weight gain (5). Fasting and postprandial plasma samples were obtained during the course of the initial unsupplemented wheat period, during the lysine-supplemented period, and again after the supplement had been withdrawn. It was then possible to evaluate the postprandial changes in plasma AA when a 44 to 50% wheat diet was obviously inadequate in available protein, when casein supplementation of a similar 50% wheat diet was for all intents and purposes satisfying protein needs, when a 75% wheat diet was marginally adequate or inadequate, when the same diet was de presumably adequate by the addition of 0.2% lysine to the wheat, and again when the lysine was withdrawn.

METHODS

In all cases plasma samples were obtained immediately before and three and four hours after the first morning meal, following an 8-hour fast, between the 9th and 101st day of consumption of each diet. The

compositions of the diets have been reported in detail, as have their digestibilities and their effects on N retention and growth[2] (4). The methods used for plasma AA analysis have also been reported (2).

The first set of samples (Table 1) was obtained from 16 infants and small children on the 9th day of consumption of a diet (a) in which 82% extraction flour from Argentine hard winter wheat supplied 44 to 50% of calories and all of 6.6% protein calories. As in all studies, the total calorie intake for each child was that which had previously been shown to support rapid weight gain when cow milk was the source of protein. Apparent N retention from this diet was 20.4±5.8% of intake, whereas it was 37.6±6.8% during an immediately preceding and 42.4±5.5% during an immediately following isonitrogenous casein diet. Despite excellent digestibility this wheat diet was obviously inadequate in utilizable protein (4).

The second set of samples (Table 2) was obtained from four infants and children on the 90th to 101st day of consumption of a diet (b) in which the same 82% extraction wheat flour provided 50% of total calories and 77 to 80% of diet protein, with enough casein, 20 to 23% of protein, to make a total of 8% protein calories. These four subjects demonstrated no evidence of malabsorption, had progressively increasing N retentions, and grew in body length and in weight at markedly accelerated rates, suggesting that neither total protein nor any specific amino acid was limiting their growth[2].

The third set of samples (Table 3) was obtained from 10 infants and small children on the 29th to 90th day of consumption of a diet (c) in which the same wheat flour provided 75% of total calories and 100%

of 9.8% protein calories. In one child the diet was well absorbed and supported satisfactory growth rates with no clear evidence of protein deficiency; the effect of lysine supplementation was never tested. In a younger infant there was minimal evidence of malabsorption, satisfactory weight gain, inadequate linear growth and some evidence of protein inadequacy; lysine supplementation was not tested. In the remaining infants and children there was more evidence of malabsorption and of protein deficiency, with growth rates that varied from clearly inadequate to marginally adequate, and it was possible to demonstrate subsequently a positive effect of lysine supplementation on N retention and weight gain[2].

The fourth set of samples (Table 4) was obtained from 7 of the above 10 infants and children 9 to 51 days after the addition of lysine to their diet (d).

The last set of samples (Table 5) was obtained from 5 of the above 7 infants and children 16 to 30 days after the supplementary lysine was withdrawn (diet e), with a resultant fall in the rate of weight gain but no significant effect on apparent N retention, serum albumin or serum cholesterol[2].

Comparisons between fasting and postprandial AA levels were made by a "t" test for paired values. Comparisons between fasting levels from different diets were made by the "t" test for paired values when the same subjects received both diets or by an independent "t" test when some or all of the subjects were different.

RESULTS

Table 1 summarizes the fasting and postprandial plasma free AA levels of infants consuming a clearly protein-deficient wheat diet. Three hours postprandially there was a significant rise in total amino acid (TAA) molar concentration, with some return toward fasting levels at four hours. The fraction of TAA represented by total essentials and semi-essentials (TEAA) was significantly lower than fasting at both three and four hours. For the eight essential and two semi-essential (cystine and tyrosine) AA concentrations (expressed as EAA/TEAA, each amino acid's molar fraction of TEAA), the most striking findings were in the lysine, isoleucine, tyrosine, phenylalanine and threonine molar ratios. Mean fasting Lys/TEAA was only one standard deviation (S.D.) below the mean found with milk protein diets at a similar % protein calories (5). Postprandially, however, there was a highly significant fall at three hours, sustained at four hours. The fasting Thr/TEAA ratio was highly variable, with the mean almost one S.D. above that found with milk protein; a modest postprandial fall became significant ($P<0.05$) only at four hours. Although fasting Ile, Tyr, and Phe/TEAA molar ratios were not significantly different from those found with milk protein diets, there were moderate but highly significant ($P<0.001$) increases in all three molar ratios at both three and four hours.

The addition of a small amount of casein to the above wheat diet, enough to raise protein calories from 6.6 to 8.0%, resulted in different plasma AA changes (Table 2). Those in TAA were not different, nor were those in TEAA/TAA ratios. The fasting Lys/TEAA ratio was significantly ($P<0.05$) greater, but there was still a highly significant fall at three and four hours: the mean ratios reached were moderately but not significantly

higher than in the previous study. Fasting Thr/TEAA ratio was conversely much lower ($P<0.001$) and there was no postprandial fall. Fasting Ile, Tyr and Phe/TEAA ratios were very similar to the previous and the 3-hour elevations were more modest, with a tendency to return to fasting levels at four hours.

When wheat provided 75 instead of 50% of calories and all of 9.8 instead of 6.6% protein calories, and the duration of feeding prior to the plasma AA studies was much longer, and signs of protein inadequacy, though generally present, were not as obvious[2], the postprandial elevation in TAA was more pronounced and there was a significant increase instead of a fall in postprandial TEAA/TAA ratios (Table 3). Fasting Lys/TEAA ratios were lower though not significantly so, and their highly significant postprandial fall reached levels which were also lower. Fasting Thr/EAA ratios were similarly high and the postprandial fall just significant ($P<0.05$) at three hours, not at four. Postprandial elevations in Ile, Tyr and Phe/TEAA ratios (Table 3) were nearly identical to those found with the 50% wheat diets (Table 1).

When lysine was added to the previous diet and resulted in significantly improved N retention and growth, with correction of indicators of protein inadequacy, there were again some marked differences in the fasting AA and in postprandial changes (Table 4). Fasting TAA were slightly higher but the postprandial elevations were more modest, neither difference being significant. Fasting TEAA/TAA ratios were higher but not significantly so, and the postprandial elevations were similar, being significant ($P<0.01$) only at four hours. Fasting Lys/TEAA ratios were now significantly ($P<0.05$ by "t" test for 7 paired values) higher

than before the addition of lysine to the wheat, and very similar to those of milk protein diets (5); there was no postprandial fall. Fasting Thr/TEAA ratios were considerably lower ($P<0.01$ by independent "t" for all values, $P<0.02$ for 7 pairs) but there was no significant postprandial change. The postprandial increases in Ile/TEAA ratios were modest and non-significant, there were no postprandial changes in Tyr/TEAA ratios, and the postprandial increase in Phe/TEAA was significant ($P<0.01$) only at three hours, this being the only significant postprandial change in any of the EAA/TEAA ratios.

When added lysine was subsequently withdrawn from the same diet there was a significant decrease in the rate of weight gain but no demonstrable change in apparent N retention and no change in other indicators of protein adequacy, suggesting that with advancing age and state of recovery the lysine deficiency of the wheat diet was becoming less important[2]. In the five children in whom plasma AA studies were carried out (Table 5) fasting TAA were higher than in previous studies (not significantly so) but there was still the typical postprandial elevation. The postprandial increase in TEAA/TAA ratios was more modest. Fasting Lys/TEAA ratios were the lowest encountered ($P<0.05$ when compared to supplemented diets) and their postprandial decline, though reaching levels comparable to those found earlier (Table 3), was not significant partly because of the lower fasting levels and partly because of the small "n". Fasting Thr/TEAA ratios were again elevated ($P<0.05$ when compared to lysine-supplemented periods) to the levels seen in the two studies without supplementation (Tables 1 and 3). A non-significant fall at three hours was followed by a return to fasting levels at four

hours. There was no change in Ile/TEAA ratios postprandially and the increases in Tyr/TEAA and Phe/TEAA did not reach significance (small "n").

DISCUSSION

The expected postprandial elevations in total plasma free AA levels were seen with all diets in this study. The relatively low fasting TEAA/TAA ratios observed with all of the diets, when compared to those seen with milk protein diets (5), probably reflect the prolonged consumption of vegetable protein with a characteristically low TEAA/TAA ratio. That these low ratios may in part be due to protein deficiency is suggested by their moderately higher values when casein, but particularly lysine, was added to the wheat diets and available diet protein became adequate for presumably maximal body protein synthesis and growth. In the subjects consuming the 6.6% all-wheat protein diet (Table 1), obviously deficient in available protein, there was a postprandial fall in TEAA/TAA ratios. That this was due to a low protein intake plus protein inadequacy, and not to a further reflection of the low dietary TEAA/TAA ratio, is indicated by the failure of such a decline to occur when the same wheat provided 9.8% protein calories (Tables 3 and 5) or when better balanced rice protein was fed (3). The number of children receiving the wheat plus casein 8% protein diet was too small to determine if the modest postprandial decline in TEAA/TAA represented a significant trend (Table 2). When lysine was added to the 9.8% wheat protein diet the postprandial elevation in this ratio may have been accentuated. When dietary protein is inadequate, a fall in the TEAA/TAA ratio may be due to an increase in unessential AA concentrations as much as to a fall in the essentials (6).

In infants and children who had been consuming a diet markedly deficient in lysine for only 8 days (Table 1), the fasting plasma molar ratio for this amino acid was not significantly lower than after milk protein diets. After a minimum of 4 weeks of consumption of a less-deficient diet (Table 3), the fasting Lys/TEAA ratio was depressed to a greater degree, confirming the previous observations on the length of time necessary for a lysine-deficient diet to affect the fasting molar ratio of this amino acid (2). The addition of casein (Table 2) or of lysine (Table 4) to these diets restored the Lys/TEAA ratio to the levels found with milk protein diets (5). The lowest fasting Lys/TEAA ratios were seen in the children who had consumed unsupplemented wheat for at least 30 days, the same supplemented with lysine for another 30+ days, and then the unsupplemented wheat for another 15 to 29 days (Table 5). This suggests that body lysine pools had been depleted, incompletely replated, and then further depleted.

The marked postprandial decline in Lys/TEAA seen with all three unsupplemented wheat diets (Tables 1, 3, 5) was undoubtedly the combined result of the low level of lysine in the diet and of its maximal incorporation into body proteins in the presence of an excess of the other essential amino acids, of unessential nitrogen, and of dietary energy. No such decline occurred when 0.2% lysine was added to the wheat (Table 4). The postprandial decline seen when casein was added was somewhat surprising as there was reason to believe that lysine was not limiting protein synthesis and growth[2]. Because the addition of lysine to this diet was not evaluated it cannot be assumed that it would not have produced a further increment in N retention, weight gain and growth.

It is possible, however, that the decline in Lys/TEAA ratios was due only to the fact that lysine, though not limiting, was still the essential amino acid in shortest supply in an 80:20 wheat protein-casein mixture. In an earlier study a 67:33 wheat protein-casein mixture, at 12.0 instead of 8.0% protein calories, did not produce a consistent fall in Lys/TEAA after a single feeding (2).

Threonine is the next-limiting amino acid, after lysine, in wheat proteins; the changes observed in its molar ratio bear this out. When lysine was clearly limiting, the fasting Thr/TEAA ratios were quite variable but on the average moderately higher than in infants consuming milk protein diets, suggesting some increase of threonine body pools. The slight postprandial decline (Tables 1, 3, 5) was probably due to its relatively short supply in wheat protein. When casein was added to wheat (Table 2), fasting Thr/TEAA was significantly lower and there was no postprandial decline. The same was true when lysine was added (Table 4), suggesting that the amount of lysine added, though preventing an accumulation of threonine in body pools, was not enough to make this amino acid clearly first-limiting.

Postprandial elevations from normal fasting levels in Ile, Tyr and Phe/TEAA ratios were seen most markedly with the clearly lysine-deficient diets. With the 50% diets these represent both real and relative increases in concentration, as the postprandial decline in TEAA/TAA ratios was due to a greater postprandial increase in non-essential than in essential AA concentrations, in part due to the low TEAA/TAA ratio in wheat protein and in part to inadequacy of available dietary protein. With the unsupplemented 75% wheat diets, more nearly adequate in available protein, there was an increase rather than a decrease in

plasma TEAA/TAA ratios, making the increases in Ile, Tyr and Phe molar concentrations more striking than those in their molar ratios of TEAA. A sharp decline in Lys/TEAA ratios should naturally be associated with an increase in the ratios for some or all of the remaining essentials. When its decline was prevented by the addition of lysine to the wheat flour, the only significant postprandial increase was in the Phe/TEAA ratio: the modest increase in Ile/TEAA ratio was not significant and there was none in the Tyr/TEAA ratio. When the lysine deficiency of wheat was partially corrected by the addition of casein, but not enough to prevent a postprandial decline in Lys/TEAA, there were still significant increases in all three ratios, most striking for Phe/TEAA. Taken together these changes suggest that there is a relative excess of these three essential amino acids in wheat protein or that it is more difficult to clear them from body amino acid pools. In the case of phenylalanine the excess or the delay in clearing it seem more important than for isoleucine or tyrosine.

These studies permit some assumptions about the potential usefulness of plasma free amino acid measurements in determining the adequacy of dietary protein and of individual amino acids.

1. A low TEAA/TAA ratio in fasting plasma can be due to the prolonged consumption of exclusive or nearly-exclusive vegetable protein and can also be caused or accentuated by a low protein intake (6), poor or delayed protein absorption (3), and protein or specific AA deficiency (this study).

2. A particularly low concentration of one essential AA, or of its molar fraction of all the essentials, in fasting plasma is indicative

of prolonged consumption of a diet in which it is present in least concentration (relative to requirements) and in which it is probably the first-limiting factor to body protein synthesis.

3. Fasting plasma AA concentrations are not likely to identify the second-limiting AA in the diet. In the case of threonine its molar ratio might actually be elevated. The addition of the first-limiting AA to the diet will after some time result in a depression of the fasting level of the previously second-limiting AA, now become first-limiting.

4. Relative excesses of other essential AA are not likely to be recognized in fasting plasma.

5. Elevations in total AA concentrations are the rule after meals containing well-digested and absorbed proteins.

6. TEAA/TAA ratios can decline postprandially if the diet protein is of predominantly vegetable origin and the % protein calories is marginal or low. Higher protein intakes of the same origins will prevent this decline, as will correction of specific AA deficiencies in the diet.

7. When a diet deficient in a specific AA has been consumed for a week or longer there will be a sharp postprandial decline in its EAA/TEAA ratio, even when the fasting ratio is already low. This is probably the most sensitive way of rapidly identifying the first-limiting AA in the diet: a fasting and a 3-hour postprandial plasma sample should be quite adequate for this purpose.

8. Postprandial changes in the plasma concentration or EAA/TEAA ratio of the second-limiting AA in the diet are not likely to be diagnostic, at least in the case of threonine.

9. Moderate but significant elevations in the postprandial plasma EAA/TEAA ratios of certain essential AA, particularly phenylalanine, are apparently indicative of their relative excesses in dietary protein or of relatively greater delays in clearing them from plasma. A real excess, as in the case of leucine in maize (7), will cause very striking postprandial elevations.

FOOTNOTES

[1] These studies were supported by Grant HD 10111 from the National Institutes of Health, U.S.P.H.S., by Contract ta-C-1286 with the Agency for International Development, U.S. Department of State, and by a grant from the Middendorf Foundation, Baltimore, Maryland.

[2] Graham, G.G., MacLean, W.C., Jr. & Lopez de Romaña, G. (19) Prolonged consumption of wheat-based diets by infants, with and without casein or lysine supplementation. Submitted to J. Nutr.

LITERATURE CITED

1. Graham, G.G., MacLean, W.C., Jr. & Placko, R.P. (1976) Plasma amino acids of infants consuming soy proteins with and without added methionine. J. Nutr. 106, 1307-1313.

2. MacLean, W.C., Jr., Placko, R.P. & Graham, G.G. (1977) Postprandial plasma lysine as an indicator of dietary lysine adequacy in infants. J. Nutr. 107, 567-573.

3. MacLean, W.C., Jr., Placko, R.P. & Graham, G.G. (1979) Postprandial plasma free amino acid changes in preschool children consuming exclusively rice protein. J. Nutr. 109, 1285-1289.

4. MacLean, W.C., Jr., Lopez de Romaña, G., Klein, G.L., Massa, E., Mellits, E.D. & Graham, G.G. (1979) Digestibility and utilization of the energy and protein of wheat by infants. J. Nutr. 109, 1290-1298.

5. MacLean, W.C., Jr., Placko, R.P. & Graham, G.G. (1978) Fasting plasma free amino acids of infants and children consuming cow milk proteins. J. Hopkins Med. J. 142, 147-151.

6. Snyderman, S.E., Holt, L.E., Jr., Norton, P.M., Roitman, E. & Phansalkar, S.V. (1968) The plasma aminogram. I. Influence of the level of protein intake and a comparison of whole protein and amino acid diets. Pediatr. Res. 2, 131-144.

7. Graham, G.G., Placko, R.P. & MacLean, W.C., Jr. (1980) Nutritional value of normal, opaque-2 and sugary-2 opaque-2 maize hybrids for infants and children. 2. Plasma free amino acids. J. Nutr. 110, 1070-1075.

Table 1

Fasting and postprandial plasma free amino acids of 16 infants and small children on the 9th day of consuming a diet (a) in which wheat provided 44 to 50% of total calories and 100% of protein (6.6% of calories). Total amino acids (TAA), total essentials (TEAA), and individual essential amino acids (EAA).

	TAA µmole/L	TEAA/ TAA	1000 x molar fraction of EAA/TEAA									
			Thr	Val	½ Cys	Met	Ile	Leu	Tyr	Phe	Lys	Trp
Fasting ±S.D.	2236 ±359	0.244 ±0.028	183 ±39	220 ±21	32 ±12	29 ± 8	58 ±10	125 ±19	73 ±24	70 ±13	124 ±24	86 ±14
3-Hour ±S.D.	2625[a] ±404	0.233[c] ±0.022	174 ±46	211 ±23	33 ±12	29 ± 7	73[a] ±15	133 ±21	98[a] ±21	93[a] ±18	75[a] ±28	80 ±14
4-Hour ±S.D.	2389[d] ±409	0.236[b] ±0.026	166[c] ±42	211 ±22	35 ±12	30 ± 7	71[a] ±11	128 ±21	102[a] ±26	93[a] ±20	78[a] ±23	86 ±14

[a] Significantly different from fasting, $P<0.001$.

[b] Significantly different from fasting, $P<0.01$.

[c] Significantly different from fasting, $P<0.05$.

[d] Significantly different from 3-hour, $P<0.01$.

Table 2

Fasting and postprandial plasma free amino acids of 4 infants and small children on the 90th to 101st day of consuming a diet (b) in which wheat provided 50% of total calories and 77 to 80% of protein, with casein added to make protein calories 8% of total.

	TAA µmole/L	TEAA/ TAA	1000 x molar fraction of EAA/TEAA									
			Thr	Val	½ Cys	Met	Ile	Leu	Tyr	Phe	Lys	Trp
Fasting	2271	0.258	99	246	37	27	68	139	77	67	153	88
±S.D.	±326	±0.026	±28	± 2	±16	± 7	±11	±15	±20	± 6	±11	±24
3-Hour	2625	0.248	97	235	35	31	87[a]	147	93	92[a]	101[a]	82
±S.D.	±472	±0.026	±24	± 4	± 6	± 9	±14	±20	± 9	± 3	±25	±15
4-Hour	2481	0.249	105	245	36	33	78	145	88[b]	86[b]	110[a]	77
±S.D.	±208	±0.029	±19	±11	± 9	±10	±25	± 6	±20	±12	±18	±10

[a] Significantly different from fasting, $P<0.01$.

[b] Significantly different from fasting, $P<0.05$.

Table 3

Fasting and postprandial plasma free amino acids of 10 infants and small children on the 29th to 90th day of consuming a diet (c) in which wheat provided 75% of total calories and 100% of protein (9.8% of calories)

	TAA µmole/L	TEAA/ TAA	1000 x molar fraction of EAA/TEAA									
			Thr	Val	½ Cys	Met	Ile	Leu	Tyr	Phe	Lys	Trp
Fasting ±S.D.	2282 ±450	0.239 ±0.022	172 ±34	215 ±23	53 ±13	32 ±7	64 ±10	138 ±22	75 ±17	74 ±13	105 ±46	72 ±15
3-Hour ±S.D.	2879[b] ±595	0.268[a] ±0.017	147[c] ±25	213 ±19	48 ± 9	35 ±5	83[b] ± 8	157[c] ±12	99[a] ±12	94[b] ±18	56[b] ±16	70 ±13
4-Hour ±S.D.	2618[c] ±540	0.273[a] ±0.020	151 ±26	217 ±22	49 ± 9	32 ±6	76[b] ±12	150 ±26	98[b] ±17	90[c] ±14	59[a] ±22	78 ±15

[a] Significantly different from fasting, $P<0.001$.

[b] Significantly different from fasting, $P<0.01$.

[c] Significantly different from fasting, $P<0.05$.

Table 4

Fasting and postprandial plasma free amino acids of 7 infants and small children on the 9th to 51st day of consuming a diet (d) in which wheat provided 75% of total calories and 100% of protein, with 0.2% lysine added to the wheat flour. This diet period followed one (c) in which no supplementary lysine was added (Table 3).

	TAA µmole/L	TEAA/ TAA	1000 x molar fraction of EAA/TEAA									
			Thr	Val	½ Cys	Met	Ile	Leu	Tyr	Phe	Lys	Trp
Fasting	2396	0.258	114	211	39	32	72	132	109	72	132	87
±S.D.	±734	±0.040	±31	±26	± 9	± 8	±18	±21	±22	±16	±44	±13
3-Hour	2654	0.269	105	196	38	32	80	142	101	93[a]	133	79
±S.D.	±453	±0.038	±17	±17	±11	± 9	±11	±21	±13	± 8	±46	±13
4-Hour	2416	0.287[a]	110	204	40	31	81	136	108	86	127	77
±S.D.	±428	±0.032	±20	± 8	±10	±10	±10	±16	±18	±15	±23	±10

[a] Significantly different from fasting $P<0.01$.

Table 5

Fasting and postprandial plasma free amino acids of 5 infants and small children on the 16th to 30th day of consuming a diet (e) in which wheat again provided 75% of calories and 100% of protein. This diet period followed one (d) in which lysine had been added (Table 4).

	TAA µmole/L	TEAA/ TAA	1000 x molar fraction of EAA/TEAA									
			Thr	Val	½ Cys	Met	Ile	Leu	Tyr	Phe	Lys	Trp
Fasting	2877	0.235	164	219	43	38	81	146	81	81	75	70
±S.D.	±522	±0.019	±40	±21	±8	± 6	±12	±28	± 8	±11	±20	±17
3-Hour	3219	0.251	144	222	43	42	81	158	93	92	56	69
±S.D.	±423	±0.022	±26	±14	±4	± 8	± 7	±11	±15	±16	±12	± 9
4-Hour	3140	0.243	170	215	44	36	74	147	93	90	57	74
±S.D.	±424	±0.036	±34	±11	±8	±10	±17	±17	±15	±19	±10	± 6