Plasma Amino Acids of Infants Consuming Soybean Proteins With and Without Added Methionine

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ABSTRACT Fasting plasma free amino acids were determined in 54 convalescent malnourished infants; seven infants while consuming a diet based on isolated soybean protein, containing 4.0% to 5.3% of dietary metabolizable energy (calories) as protein (A), 20 at 6.4% to 6.7% protein calories (B), 23 at 6.4% to 6.7% protein calories with added L-methionine (C), and four with 8.0% to 12.3% protein calories (D). There were no differences in total amino acid concentration (TAA) among the four groups; the molar fraction of essential amino acids (EAA:TAA) was lower for group A; there were no differences among the four groups in Lys:EAA or \( \frac{1}{2} \) cystine:EAA ratios or in Met concentration. Met:EAA was higher in C than B, with considerable overlap of individual values. In 10 of 13 infants who were represented in both B and C, Met concentration and Met:EAA ratio were higher in group C. Fasting plasma AA levels are not consistently reliable for field or clinical assessment of dietary Met adequacy. Fasting and postprandial (3- and 4-hour) plasma AA were determined in 29 infants: in 12 the preceding diet and the test meal were both Met-deficient with <6.7% protein calories (E), in five the preceding diet was milk-based but the test meal was Met-deficient at <6.7% (F), in five the preceding diet and test meal were based on isolated soybean protein at <6.7% with L-Met added (G), and in seven the test meal was soy-based with >9.0% protein calories (H). Plasma Met concentration and Met:EAA fell significantly at 3 and 4 hours in groups E and F, but not in groups G and H, suggesting that a postprandial fall in Met:EAA ratio can be used to identify dietary Met deficiency in field situations. J. Nutr. 106: 1307-1313, 1976.

INDEXING KEY WORDS plasma amino acids · methionine intake

In the evaluation of human diets, it is of considerable practical significance to be able to determine if one of the essential amino acids is limiting for protein synthesis. Diets which are reputed first-limiting in the sulfur-containing amino acids are consumed in many parts of the developing world (1). Soybean protein, which is notably deficient in methionine (Met), is being used more and more in the diet of the affluent countries. It is important in both instances to know if this amino acid is the limiting factor in the diet and if methionine supplementation is indicated.

We previously indicated that the fasting

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levels of plasma free methionine did not distinguish readily between infants who were consuming diets which were deficient in this amino acid and those which were not (2). In a small number of infants, we found that postprandial plasma methionine, particularly when expressed as the molar fraction of the essential acids, did distinguish between those consuming Met-deficient and Met-adequate diets (3). This was apparent by a fall in its molar fraction in plasma, notable at 3 hours and even more so at 4 hours after a test meal deficient in methionine. We indicated that additional experience was needed with the 4-hour sample and with "corrected" diets.

We are now reporting additional experience with fasting and postprandial plasma amino acid determinations in infants consuming diets which were clearly deficient in methionine, "corrected" by the addition of DL-methionine, or "corrected" by increasing the level of intake of the deficient protein so that methionine was presumably no longer limiting.

MATERIALS AND METHODS

These studies were carried out over a 6-year span in infants and small children, 5 to 42 months of age, who were well advanced in their convalescence from malnutrition, having reached a body weight which was at least 90% of that expected for their body length, and a serum albumin level of at least 3.8 g/100 ml, the lower limit of normal for our laboratory. The protocols were approved by the Joint Committee on Clinical Investigation of the School of Medicine, The Johns Hopkins University, and by the Committee on Human Research of the Instituto de Investigacion Nutricional, Lima, Peru. Informed consent was obtained from the parents of all subjects.

In 54 studies (table 1), fasting plasma free amino acids were determined after a minimum of 3 days of consuming the specified diet. Energy intakes were 90 to 175 kcal/kg body weight/day, individually determined as sufficient to maintain a rate of weight gain of 3 to 5 g/kg body weight/day. Vitamin and mineral supplements at all times met the recommended dietary allowances for age (4).

In seven of the 54 fasting studies, total dietary metabolizable energy (calories) as protein was 4.0% to 5.3% (group A), below the 6.4% which is generally considered the lower limit of adequacy for infants consuming diets based on high quality protein (5). In six of these seven studies the sole source of protein was an isolated soybean protein (ISP) and in the remaining study it was manioc flour (cassava flour with 1.8% of energy as protein) enriched.

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**TABLE 1**

Fasting plasma free amino acids of infants consuming diets whose protein component had methionine as the first-limiting factor. In 43 diets soybean was the sole source of protein; in 11 diets it was a mixture of soybean and another protein.

<table>
<thead>
<tr>
<th>Group</th>
<th>Calories as protein</th>
<th>n</th>
<th>TAA</th>
<th>EAA/TAA</th>
<th>Lys/EAA</th>
<th>Cystine/EAA</th>
<th>Met</th>
<th>Met/EAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µmoles/100 ml</td>
<td></td>
<td>µmoles/100 ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4.0-5.3</td>
<td>7</td>
<td>258.7 ± 0.23</td>
<td></td>
<td>1.6 ± 0.027</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>6.4-6.7</td>
<td>20</td>
<td>254.1 ± 0.275</td>
<td></td>
<td>1.9 ± 0.027</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6.4-6.7</td>
<td>23</td>
<td>237.5 ± 0.264</td>
<td></td>
<td>2.2 ± 0.034</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>8.0-12.3</td>
<td>4</td>
<td>235.0 ± 0.276</td>
<td></td>
<td>2.2 ± 0.046</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 TAA = total plasma free amino acids. 2 EAA = total plasma free essential amino acids (+ Cys and Tyr) in µmoles/100 ml. 3 Lys/EAA = plasma free lysine as the molar fraction of EAA. 4 Mean ± SD. 5 Significantly (P < 0.01) lower than groups B and C. 6 DL-Methionine, 12-20 mg/kg body weight, added to diet. 7 Significantly (P < 0.05) higher than group B.
with 10% ISP so that it provided 86% of total protein.

In 20 studies (group B) the percentage of protein calories was 6.4% to 6.7%. In 12 of these the sole source of protein was the same ISP, in four of them the manioc flour enriched with ISP, in one of them a toasted soybean flour mixture diluted with nonprotein calories to the desired percentage, and in the remaining three studies a mixture of casein 60% and ISP 40%.

In 23 studies (group C) the percentage of protein calories was also 6.4% to 6.7%, but n-Met, 12 to 20 mg/kg/day, was added to the formula feedings, an amount estimated to correct the methionine deficit. In 20 of these studies, the sole source of protein was the ISP, in two of them it was the ISP-enriched manioc, and in the remaining one it was the diluted toasted soy flour formula.

In the last four studies (group D) the percentage of protein calories was 8.0%, 9.1%, 10.0%, and 12.3%, respectively. In the first one, the source of protein was the casein-ISP mixture and in the remaining three it was ISP alone. In each case it was estimated that the intake of sulfur-containing amino acids was enough to satisfy the requirement without recourse to amino acid supplementation.

Blood was drawn for analysis in the morning after a fast of 8 hours. Plasma free amino acids were determined by ion-exchange column chromatography (6) with the exception of tryptophan, which was determined fluorometrically (7).

In 29 additional studies (table 2), blood was obtained in the morning after an 8 hour fast, immediately before consuming the first feeding of the day, and again 3 and 4 hours after completing its consumption.

In 12 of these studies (group E), the percentage of calories from protein was less than 6.7%, the first feeding of the day of study (test meal) was of the same composition as the diet of the preceding three days, and ISP was the only source of protein.

In five studies (group F), the percentage of calories from protein was less than 6.7% in the test meal, which had ISP as its only source of protein, but the diet of the preceding three days had cow milk as the only source of protein at 8.0% or more of calories and was thus clearly not limiting in the sulfur-containing amino acids.

In a further five studies (group G), the

### TABLE 2
Fasting and postprandial (3- and 4-hour) plasma free amino acids in infants receiving a test meal whose protein had methionine as the first-limiting factor

<table>
<thead>
<tr>
<th>Group</th>
<th>Met in diet protein</th>
<th>% Calories as protein</th>
<th>Time of meal</th>
<th>EAA/TAA</th>
<th>EAA</th>
<th>Met/EAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>Deficient</td>
<td>&lt;6.7</td>
<td>12 fast</td>
<td>0.354</td>
<td>0.025</td>
<td>0.0322</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 hr</td>
<td>0.267</td>
<td>0.026</td>
<td>0.0222</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 hr</td>
<td>0.275</td>
<td>0.039</td>
<td>0.0595</td>
</tr>
<tr>
<td>F</td>
<td>Adequate</td>
<td>&lt;6.7</td>
<td>5 fast</td>
<td>0.390</td>
<td>0.018</td>
<td>0.0066</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 hr</td>
<td>0.411</td>
<td>0.024</td>
<td>0.0091</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 hr</td>
<td>0.331</td>
<td>0.010</td>
<td>0.0107</td>
</tr>
<tr>
<td>G</td>
<td>Deficient</td>
<td>&lt;6.7</td>
<td>5 fast</td>
<td>0.243</td>
<td>0.018</td>
<td>0.0066</td>
</tr>
<tr>
<td></td>
<td>Met added</td>
<td></td>
<td>3 hr</td>
<td>0.247</td>
<td>0.009</td>
<td>0.0068</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 hr</td>
<td>0.258</td>
<td>0.009</td>
<td>0.0044</td>
</tr>
<tr>
<td>H</td>
<td>6 Adequate</td>
<td>&gt;9.0</td>
<td>7 fast</td>
<td>0.309</td>
<td>0.033</td>
<td>0.0028</td>
</tr>
<tr>
<td></td>
<td>1 Deficient</td>
<td></td>
<td>3 hr</td>
<td>0.302</td>
<td>0.019</td>
<td>0.0066</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 hr</td>
<td>0.312</td>
<td>0.009</td>
<td>0.0038</td>
</tr>
</tbody>
</table>

*Significantly (P < 0.05) different from fasting value for same diet.  
Significantly (P < 0.02) different from fasting value for same diet.  
Significantly (P < 0.01) different from fasting value for same diet.  
Significantly (P < 0.001) different from fasting value for same diet.
percentage of calories from protein was less than 6.7% in both the test meal and the preceding diet and both had ISP enriched with DL-Met as the only source of protein.

In the last seven studies (group H), the percentage of protein calories was 9.1% to 12.3%. In all of them the test meal had ISP as the only source of protein; in one the protein source of the preceding diet was the same, and in the other six it was either cow milk or a mixed diet.

RESULTS

Fasting studies. Table 1 summarizes the pertinent results of these studies. The concentration of total plasma free amino acids was not different for the three levels of protein calories and methionine supplementation did not affect it. The ratio of the sum of concentrations of the eight essential and two "semiessential" amino acids (EAA) to that of total amino acids (TAA) in the 4.0% to 5.3% protein group (A) was significantly lower by an unmatched "t" test (8) than that of either of the 6.4% to 6.7% diets (B and C). There were no differences in the EAA:TAA ratios among the unsupplemented 6.4% to 6.7% diets (B), the supplemented 6.4% to 6.7% diets (C), and the 8.0% to 12.3% diets (D). The concentrations of 1/2 cystine, as the molar fraction of the EAA, were not different among the four groups. The actual molar concentrations of methionine were also not significantly different among the four groups, although the difference between the unsupplemented (B) and supplemented (C) 6.4% to 6.7% diets was suggestive \( (t = 1.76, P < 0.1) \). When the molar fractions of Met:EAA were compared, the difference between those two groups was significant. None of the differences for the molar fractions of lysine were significant. The results for this amino acid are included as illustrative of the changes in the other essential amino acids.

In the 6.4% to 6.7% protein studies, there were 13 infants who at different times with preceding and intervening periods during which they consumed milk protein, received ISP diets with and without added methionine, which were otherwise identical. This allowed comparisons by a paired "t" test (8). For the unsupplemented and supplemented diets, respectively, the values for TAA were 246.3 ± 42.4 and 238.6 ± 30.9 \( \mu \)moles/100 ml (mean ± sd); for EAA:TAA they were 0.276 ± 0.033 and 0.271 ± 0.048; for \( 1/2 \) cystine:EAA they were 0.033 ± 0.017 and 0.036 ± 0.014; for methionine concentration they were 1.5 ± 0.5 and 2.2 ± 0.5 \( \mu \)moles/100 ml \((t = 2.812, P < 0.02)\); for Met/EAA they were 0.027 ± 0.006 and 0.035 ± 0.009 \((t = 2.606, P < 0.05)\); and for Lys/EAA they were 0.174 ± 0.030 and 0.170 ± 0.032. Only the differences in methionine, either as its actual concentration or as its molar fraction of EAA, were significantly different. There were two instances in which the methionine concentration was the same after the unsupplemented and supplemented diets and one in which it was higher after the unsupplemented diet. In three instances the molar fraction was higher after the unsupplemented diet.

Postprandial studies (table 2). For the 12 studies (E) in which both the preceding diet and the test feeding were clearly limiting in methionine (and total S-containing amino acids), TAA were significantly higher (by paired "t" test) at 3 hours than fasting, and significantly lower \((P < 0.02)\) at 4 hours than at 3 hours. Differences in EAA/TAA, \( 1/2 \) cystine/EAA, and Lys/EAA were not significant, but the Met/EAA ratio at 3 hours was significantly lower than fasting, as was that at 4 hours. In 9 of 12 instances, the 3-hour Met/EAA ratio was lower than the fasting, in 11 of 12 the 4-hour ratio was lower than fasting, and in 8 of 12 both were lower. In all 12, at least one of the two postprandial ratios was lower than the fasting ratio.

For the five studies (F) in which the preceding diet was milk-based, higher in protein content, and adequate in methionine, and the test meal was limiting in methionine, there were no significant changes at 3 or 4 hours in TAA concentration, EAA/TAA ratio, or Lys/EAA ratio. There were, however, significant falls in the \( 1/2 \) cystine/EAA ratio at 3 hours and 4 hours, when compared to the fasting ratio. In all five instances, both the 3- and 4-hour ratios were lower than the fasting. Plasma methionine concentrations at 3 and 4 hours (not included in the table) were always lower than the fasting concentration. The Met/EAA ratio at 3 hours was
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significantly lower than the fasting ($t = 2.85, P < 0.05$); the ratio at 4 hours even more so ($t = 10.1, P < 0.001$).

For the five studies (C) in which both the preceding diet and the test meal yielded less than 6.7% protein calories from ISP supplemented with methionine, there was again a significant increase in TAA at 3 hours and a fall, not significant, at 4 hours. Changes in EAA/TAA, $\frac{2}{3}$ cystine/EAA, and Lys/EAA ratios were not significant. There was a significant increase in the Met/EAA ratio at 3 hours and at 4 hours, with all values above the fasting ratio. The same was true for methionine molar concentration.

In the last group (H), those receiving more than 9.0% protein calories from un-supplemented ISP, there were no significant differences in TAA, EAA/TAA, Lys/TAA, $\frac{2}{3}$ cystine/EAA or Met/EAA between the fasting and the 3- and 4-hour samples. In four of the seven the $\frac{2}{3}$ cystine/EAA ratio at 3 hours was below the fasting level, with a recovery at 4 hours, but in the remaining three it was the same as fasting or higher than fasting in six of seven instances. In one subject both the 3- and 4-hour ratios, 0.026 and 0.024, were below the fasting ratio of 0.028. The methionine molar concentration was below fasting levels in five of seven instances at 3 hours and in three of seven at 4 hours. In only one instance were both the 3- and 4-hour concentrations below the fasting level.

DISCUSSION

Under the carefully controlled conditions of a metabolic unit, it is relatively simple to demonstrate that a particular diet is limiting in lysine for rapidly growing infants (9). It is also possible, though not as readily, to demonstrate that a diet is limiting in methionine or the sulfur-containing amino acids (10). In a field situation, it is much more difficult to demonstrate that the diets being consumed by infants and children in a given population are deficient in one of the essential amino acids and are thus subject to significant improvement from specific amino acid supplementation. If it were possible to obtain fasting blood samples from a representative number of children in a study population, determine the levels of the free amino acids in plasma, and identify one of the essential amino acids as limiting protein synthesis and growth, an extremely valuable tool would be available to assess the protein quality of their diets and to plan its amelioration. The results of the fasting amino acid levels in the present study suggest that such a single sample would be of limited value, even in a large enough population.

In the rapidly growing infant, prolonged consumption of diets which provide less than 6.4% of calories as protein are likely to produce manifestations of protein deficiency, most notably a fall in serum albumin and an increase in liver fat (5, 11). At these levels, if the source of protein is one which is notably deficient in methionine, as is soybean protein, it is possible to demonstrate an increase in nitrogen retention after methionine supplementation (10). In the present study, the only significant difference between the fasting plasma amino acid levels of the seven infants consuming such diets (group A) and those of the 20 infants consuming 6.4% to 6.7% protein calories (group B), or of the 23 consuming similar diets enriched with $\alpha$-methionine (group C), or of the 4 consuming 8.0% to 12.3% protein calories (group D) was in the ratio of the molar concentration of essential and semiessential amino acids to that of total amino acids (EAA/TAA). Such a difference would be found even if the source of protein were cow milk (12) and is indicative of the fact that the protein content of the diet is low in relation to total energy.

When the diet provides 6.4% to 6.7% of energy as soybean protein, it is still possible in most infants to demonstrate a supplementary effect of methionine (10). In the present study, the only significant difference in plasma amino acid levels between 20 infants consuming 6.4% to 6.7% diets (group B) and 23 infants consuming the same with added $\alpha$-methionine (group C) was in the molar concentration of methionine when it was expressed as a fraction of the total essential and semiessential amino acids. This was not a significant difference and there was considerable overlap between the two groups of values.
Even when the results in the 13 infants who received the same diet with and without added methionine were analyzed, there were three instances in which the wrong assumption would have been made as to methionine adequacy. The plasma amino acid levels of the 4 infants (group D) receiving 8.0% to 12.3% of calories as protein (primarily soybean) were not different from those of the infants receiving 6.4% to 6.7% protein calories (groups B and C), despite the fact that at this level, the S-containing amino acids were no longer limiting by the nitrogen balance technique or in prolonged growth studies. It is possible that the slight elevation in the fasting plasma methionine of those receiving added D-Met (group C) was due to the unutilized D-isomer, which is usually excreted in the urine (13), and does not necessarily signify methionine adequacy. 

The results of the postprandial studies are much more promising of possible field or clinical use. Whereas the fasting plasma amino acid levels of the first 12 infants (group E), those who had been consuming a Met-deficient diet prior to the test meal, were not diagnostic, the postprandial fall in the Met/EAA ratio after consuming a meal of the same composition was suggestive of methionine deficiency, particularly in the 4-hour sample. This type of study would be analogous to a field or clinical situation in which an individual or group of individuals would have blood samples taken immediately before and 4 hours after consuming a typical meal. That the fall in the Met/EAA ratio represents true methionine deficiency, and not merely the fact that this was the amino acid present in the lowest concentration (relative to requirements), is suggested by the much lesser fall or lack of a fall in the Met/EAA ratio seen in the last seven infants (group H). Their test meal contained the same dietary protein, but at a concentration high enough to probably meet the methionine requirement of each child for the conditions of the study.

The second group of infants (F), those whose previous diet had at least 8% of calories as milk protein, but whose test meal was methionine-deficient at less than 6.7% protein calories, is analogous to a group of children known to have been receiving an adequate diet who then receive a test meal suspected of being deficient in a specific amino acid. In this situation both the cystine and the Met/EAA ratios were below the amino acid ratios in all samples at 3 and 4 hours. We attach considerably more significance to these ratios than to the actual molar concentrations, as a fall in these might just represent the change from a high to a marginal level of protein and essential amino acid intake. The fact that both S-containing amino acids decreased much more than the other essentials, is indicative of a lower concentration, relative to requirements. The lack of a comparable decrease in the last group of infants (H), those receiving a soybean protein test meal at more than 9.0% protein calories, lends support to the interpretation of a significant decrease in postprandial cystine/EAA and Met/EAA ratios as indicative of a dietary deficiency of the S-containing amino acids.

In all five infants (G) receiving the Met-supplemented soybean protein diet, there was an elevation of the Met/EAA ratio both at 3 and 4 hours after the test meal, as was to be expected. Some of this elevation might be due to the unusable D-isomer. Total amino acid concentration 3 hours after the test meal was significantly higher than the fasting concentration in those groups of infants (E and G) whose previous diet was just as low in protein as the test meal, less than 6.7% protein calories. In those whose previous diet was richer in protein (F and H), this postprandial elevation was not seen.

The EAA/TAA ratios of the four groups of infants were not significantly altered by the test meal, even when this was much lower in protein than the previous diet, suggesting that some days may be necessary before this indicator of dietary protein adequacy is affected by a deficient diet.

These studies suggest two ways in which the plasma amino acid levels can be used to judge the adequacy of methionine or total sulfur-containing amino acids in a particular diet for growing children. Met/EAA ratios could be determined in a small group of children of the appropriate age group (who had previously been consuming a known adequate diet) just before and 3 or 4 hours after eating a meal of the diet in question. A decrease in this ratio
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would be suggestive of methionine deficiency.

In children already consuming the diet in question, it would be preferable to obtain both the 3- and 4-hour sample, as well as the fasting sample. A decrease in the Met/EAA ratio in both postprandial samples would almost certainly indicate that methionine was limiting for protein synthesis and not just first-limiting in the dietary protein.

LITERATURE CITED
