Nitrogen Balance and Plasma Aminogram in Measuring Supplemental Effect of Amino Acids for Children

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The potential usefulness of the plasma aminogram as an indicator of dietary protein or specific amino acid adequacy has been examined. Fasting plasma lysine increases with intake in infants and children but does not identify the level of adequacy, as determined by nitrogen retentions. Postprandial levels of lysine rise on adequate intakes and fall after a lysine-deficient test meal. The ratio of essential to total amino acids in fasting plasma does not discriminate between a lysine-deficient and a corrected diet, nor does it do so for methionine-deficient diets at critical levels of protein intake. Fasting levels of plasma methionine or methionine + cystine are not affected by intakes which border on adequacy, as distinguished by the nitrogen balance technique. The determination of postprandial amino acid levels shows more promise than that of fasting levels as a tool for measuring the adequacy of specific amino acids in the diet.

Comparative nitrogen balances, carried out at appropriately critical levels of intake and with careful attention to detail, continue to be the best technique for obtaining, within a reasonable length of time, reliable estimates of dietary protein quality for infants and children. Their major disadvantage lies in the elevated costs and inconveniences entailed in the collection of quantitative urine and stool samples. When amino acid enrichment of dietary proteins is contemplated, satisfactory documentation of expected gains is necessary. For lysine- or methionine-deficient proteins we have documented significant increases in apparent nitrogen retention after appropriate supplementation (Graham *et al.*, 1969b; Graham, 1971). Such evaluations would be expedited considerably by a less cumbersome technique.

If the plasma concentrations of the major amino acids were reliable indicators of the adequacy of dietary protein or of specific essential amino acids, a technique would be available which required only careful measurement of the intake of specified diets and the drawing of appropriate blood samples. When protein intake is grossly inadequate for a significant length of time, a fairly characteristic pattern emerges, the so-called kwashiorkor pattern, whether the result of natural circumstances (Holt *et al.*, 1963) or of experimental manipulation of the diet (Snyderman *et al.*, 1968b). At the other extreme, inordinately high protein intakes produce fairly typical alterations (Snyderman *et al.*, 1968b). Between these two extremes, however, when intake hovers on the borders of adequacy, alterations in plasma levels of amino acids have been insignificant (Graham and Placko, 1970).

For most essential amino acids, complete or nearly-complete withdrawal, when the supply of others is adequate, produces a significant drop in its plasma level and, for some, characteristic alterations in the levels of other amino acids (Snyderman *et al.*, 1968a). In the course of studies of the lysine enrichment of wheat flour, we found that the fasting level of plasma free lysine did increase steadily when its intake was increased from an obviously inadequate level to an adequate one and on to a seeming excess (Graham *et al.*, 1969b). There was no apparent plateau at a presumably adequate level and there was enough individual variability for us to doubt the value of this indicator in very small groups or single cases, if used to determine the first-limiting factor in the diet. Despite these changes in plasma lysine levels, and despite significant differences in nitrogen retention, there were no differences in total amino acid levels, or in the ratio of essentials to nonessentials. This same ratio was higher for the control casein or milk diets, suggesting that at borderline intakes it is more indicative of the same ratio in the dietary protein than of the adequacy of the diet.

A recent report of the effect of different levels of tryptophan intake on its fasting and postprandial plasma level in volunteer adults revealed a different pattern (Young *et al.*, 1971). Instead of a plateau around the level of adequacy, as determined by the balance technique, or a steady rise as in our experience with lysine, there was a minimum plasma level, even at zero intake, which was not exceeded until an adequate intake was reached, at which point there was a steady rise until a new level was reached, this in turn not being exceeded after further increments in intake. The much more complex metabolic pathways for tryptophan, as well as the different conditions of the study, probably account for these differences. In chronic tryptophan- or protein-deficient states, plasma tryptophan levels are more severely depressed (Truswell *et al.*, 1968).

The present report summarizes some of our previously reported experiences with methionine-deficient and supplemented diets, as well as some limited studies of postprandial amino acid levels.

METHODS

The subjects of these studies were convalescent malnourished infants and children, and the calorie intakes those necessary for steady gains in weight. Protein intakes were those which were just adequate when milk was the source of protein. Nitrogen balances, at these borderline protein and adequate calorie intakes, were determined for the supplemented and unsupplemented protein sources. Diets were kept isonitrogenous and isocaloric per unit of body weight by appropriate reduction of the protein intake when the amino acid was added. The intakes of all other nutrients were considered to be ample (Graham *et al.*, 1969a). Fasting or postprandial plasma free amino acids were determined by ion exchange column chromatography (Spackman *et al.*, 1958).

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Table I. Total Fasting Plasma Free Amino Acids (TAA) of Children on Methionine-Deficient (Left-Hand) or Supplemented (Right-Hand Column) Diets. Number of Samples in Parentheses

Total amino acids μM/ml ± SD			
		ProSobee	2.483 ± 0.521 (12)
Sobee	2.905 (1)	Sobee + DL-methionine	2.552 (1)
Supro	2.110 ± 0.069 (2)	Supro + DL-methionine	2.351 ± 0.189 (7)
GF1	2.087 ± 0.402 (9)	GF2	2.029 ± 0.219 (7)
CSM	2.098 ± 0.523 (13)	CSM + DL-methionine	2.734 ± 0.240 (2)
		Fortifex	1.969 ± 0.131 (5)
Mean	2.127 ± 0.466 (25)	Mean	2.304 ± 0.410 (34)

Table II. Ratio of Essential to Total Fasting Plasma Amino Acids (EAA/TAA) of Children Receiving Methionine-Deficient and DL-Methionine-Supplemented Diets. Same Samples as in Table I

Essential amino acids/total amino acids			
		ProSobee	0.270 ± 0.040 (12)
Sobee	0.330 (1)	Sobee + methionine	0.237 (1)
Supro	0.330 ± 0.006 (2)	Supro + methionine	0.336 ± 0.067 (7)
GF1	0.288 ± 0.043 (9)	GF2	0.292 ± 0.032 (7)
CSM	0.259 ± 0.052 (13)	CSM + methionine	0.305 ± 0.087 (2)
		Fortifex	0.266 ± 0.023 (5)
Mean	0.278 ± 0.051 (25)	Mean	0.289 ± 0.051 (34)

Table III. Fasting Plasma Free Methionine, Methionine + Half Cystine, and Methionine + Half Cystine + Taurine of Children on Methionine-Deficient and Supplemented Diets. Molar Concentration and Molar Fraction of Total Amino Acids for Same Samples as in Table I

Plasma amino acid, fasting	Diets: Methionine deficient (n = 25)	Diets: Methionine supplemented (n = 34)
Methionine, μM/ml	0.020 ± 0.007	0.023 ± 0.008
Methionine + Half cystine, μM/ml	0.052 ± 0.047	0.049 ± 0.015
Methionine + Half cystine + taurine, μM/ml	0.111 ± 0.097	0.122 ± 0.073
Methionine/total amino acid	0.009 ± 0.002	0.010 ± 0.003
Methionine + Half cystine/total amino acid	0.024 ± 0.015	0.022 ± 0.008
Methionine + Half cystine + taurine/total amino acid	0.050 ± 0.034	0.053 ± 0.031

RESULTS

Table I summarizes the values for total fasting plasma free amino acids on a variety of diets. Those in the left-hand column correspond to sources of protein which at the levels fed were limited in their utilization by digestibility, methionine deficiency, or a possible combination of both. Those in the right-hand column represent the same sources, enriched with adequate amounts of DL-methionine, plus two other products already enriched by the manufacturer. Sobee is a soy "milk" based on toasted soy flour, Supro is an isolated soy protein, GF1 is a corn–soy–wheat macaroni, GF2 is the same enriched with methionine, CSM is a corn–soy–milk mixture, ProSobee is a soy "milk" based on a soy protein isolate enriched with methionine, and Fortifex is a corn–soy mixture enriched with methionine. For each unenriched product, methionine supplementation resulted in improved nitrogen retention. Although there was considerable variability in the total plasma AA levels, there was no consistent difference between each deficient protein and its enriched version; the difference between the mean values was not significant.

Table II summarizes the ratio of essential amino acids (tryptophan excluded) to total amino acids in the same plasma samples. Again there were no clear trends and the means were not significantly different.

Table III summarizes for the same samples the values for sulfur-containing amino acids. Whether plasma methionine was expressed as its absolute concentration or as a fraction of total amino acids, there was no difference between the deficient and the supplemented diets. When the values for methionine and cystine were combined, there was again no difference, nor was there one when values for taurine were added.

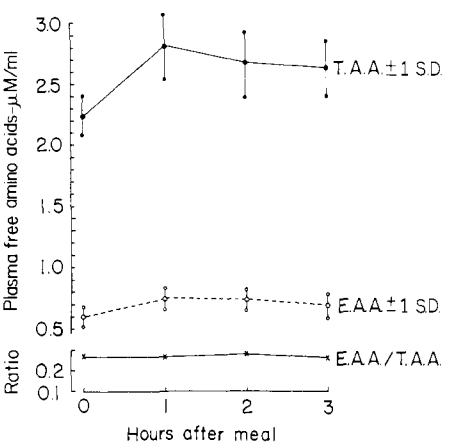

Figure 1. Postprandial plasma free amino acids in nine children receiving casein or cereal-based diets. TAA is combined value of essentials and nonessentials; EAA is essentials only, excluding tryptophan

Fasting and 1, 2, and 3-hr postprandial plasma samples were obtained in nine children receiving lysine-adequate or lysine-deficient test meals, as demonstrated by the nitrogen balance method. For some the source of protein in the test meal was the same as they had been receiving for many days, while for others it was totally different.

Figure 1 represents the evolution of the mean values (±1 SD) for total and essential amino acids (tryptophan

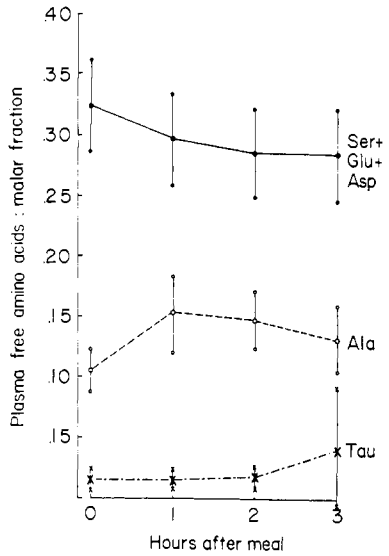

Figure 2. Postprandial plasma free amino acids in same samples as Figure 1. Values for some of the nonessentials as their molar fraction of the total

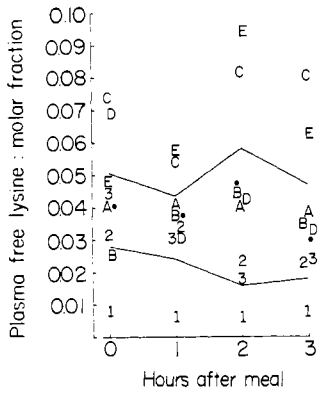

Figure 3. Postprandial plasma free lysine (molar fraction) in same samples as Figure 1. Upper line is for six test meals adequate in lysine. Individual values for same identified by letters A–E and asterisks, these last are from a lysine-enriched wheat flour diet. Lower line and numbers 1–3 are for wheat flour diets

excluded), as well as the ratio between these, which did not vary appreciably. Between the fasting (8 hr) and 1-hr samples there was a very distinct rise, with a gradual falling off in the next 2 hr for both total and essential amino acid concentrations. After 3 hr the fasting level had not been regained. Some of the amino acids did not follow the general trend (Figure 2). The combined value for serine + glutamine + asparagine declined during the first 2 hr, while that of alanine displayed a sharp rise at 1 hr and a gradual decline thereafter. Taurine did not change appreciably until 3 hr, when in some cases there was a sharp rise.

The top line in Figure 3 represents the mean plasma lysine for the six children in whom the lysine content of the test meal was known to be adequate. Individual cases are represented by the letters A–E and by the asterisks, these last corresponding to a test meal of wheat flour enriched with lysine. The drop between the fasting and 1-hr level in two cases (C and D) probably represents a decrease in intake from the preceding diet, but could also represent experimental error. In five of the six cases there was a variable rise between 1 and 2 hr postprandially. Three children (1, 2, and 3) received a test meal of unenriched wheat flour, clearly deficient in lysine. In one of them (No. 1) the preceding diet was also wheat, so that all values for lysine were strikingly low. For the other two there was a distinct drop at 2 hr, persisting until the third hour. The bottom line represents the mean for these three cases.

DISCUSSION

If the determination of plasma free amino acids is to prove useful in evaluations of the adequacy of amino acid supplementation, it should be able to discriminate between diets which are deficient in one or more amino acids and those in which the deficiency has been corrected. Our previous studies indicated that fasting levels of total and essential amino acids did not distinguish lysine-deficient from lysine-adequate wheat diets. The fasting lysine level did so, but with a degree of accuracy of probably limited usefulness.

In the present studies, when methionine was the limiting amino acid, because soy was the main or only source of protein, the total and essential fasting amino acid levels did not discriminate between these diets and the same or similar ones, in which methionine had been added and an improved retention of nitrogen demonstrated (Graham, 1971; Graham *et al.*, 1971a,b). More important, the levels of plasma methionine, or of methionine + cystine, or methionine + cystine + taurine also failed to discriminate between deficient and supplemented diets. It must be remembered, however, that the deficient diets were far from being devoid of sulfur-containing amino acids and that the level of supplementation was only the minimum considered necessary for correction.

When postprandial amino acid levels are examined, the limited number of studies does suggest that these probably discriminate more accurately between a lysine-deficient and a lysine-adequate meal. This is in line with the tryptophan studies already alluded to (Young *et al.*, 1971) and with the extensive animal studies of McLaughlan and Morrison (1968). Additional observations are needed to confirm this trend and to look for it in diets deficient in methionine.

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