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9. ABSTRACT

Fasting plasma was obtained from infants and small children who had been admitted to the British American Hospital, Lima, Peru, for the treatment of severe primary malnutrition at least six weeks previously. For at least nine days the children had been on diets whose only source of protein was a modified cow's milk or calcium caseinate, along with supplemental vitamins and minerals. Analyses showed that within a relatively narrow range of dietary protein calories, 6.4 to 8.0% of the total, neither the source of milk protein nor its level had a significant effect on fasting plasma total free amino acids, the ratio of essential to total amino acids, or the molar fraction of individual essential amino acids of the convalescent malnourished infants and small children. This level of milk protein calories was presumably adequate for maximal growth, as measured by other parameters. Minor differences in total amino acids were probably the result of differences in the efficiency of intestinal absorption. The molar fraction for lysine was suggestively affected by its level in the diet.

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Plasma Amino Acids: Milk Diets

If children gain in height and weight at the expected rate for prolonged periods of time, there is seldom reason to question the adequacy of the diet. When calorie intake is adequate or excessive but protein is not, gains in height and particularly in weight can continue for many weeks but the serum albumin levels will then decrease and hepatic steatosis eventually develops: the inadequacy of the diet may thus not be apparent for many days or weeks. In patients receiving severely restricted protein intakes, in the field evaluation of children suspected of protein malnutrition, and in the evaluation of new protein sources under consideration for general use, it would be most helpful to learn readily if a given protein intake is adequate for "normal" protein synthesis.

The recognition of marked alterations in the plasma amino acid levels of children suffering from kwashiorkor (1) has stimulated interest in their measurement as an indicator of protein nutriture. A much simplified technique, measuring the ratio of some non-essential to other essential amino acids in serum (2) has been introduced as a field tool in the measurement of the status of protein metabolism and has achieved wide acceptance. It has been noted, however, that this ratio is sensitive to recent dietary intake and that a protein-malnourished child who has been on a good diet for a short time may have a normal ratio and that a healthy child receiving a protein-deficient diet for a few days may have an abnormal ratio, thus limiting it to a measure of dietary protein adequacy and not one of protein nutriture (3). A recent publication (4) has documented

the effect of varying levels of milk protein and of an amino acid mixture simulating it on the plasma aminogram of healthy premature and term infants. As early as two days after the reduction of dietary protein to 4.0% of calories, the plasma aminogram was indistinguishable from that of a case of kwashiorkor. At 6.0% protein calories all values, with the exception of that for glycine, fell within one standard deviation of the mean for their control infants receiving 12.0% protein calories. At 32% protein calories marked alterations in plasma levels were produced by the milk protein diet, not so by the equivalent amino acid diet. In their studies the plasma aminogram was thus "resistant" to all but extreme alterations in the level of protein intake.

McLaughlan and Morrison have shown that the plasma aminogram of the rat can identify the first-limiting amino acid in a particular diet (5). Snyderman et al (6) have documented the effects of very low or very high intakes of individual amino acids on the plasma aminogram of infants. Both, however, studied extreme situations which are not likely to be encountered in practice. We are concerned with the evaluation of unconventional sources of dietary protein (7) and with amino acid supplementation of cereals (8). At equally critical levels of intake, in a group of six children receiving casein, wheat flour, or the same flour enriched with increasing levels of lysine, we found no difference in the total molar content of free amino acids in fasting plasma obtained after 9 or more days on the respective diets. After the casein diets, despite approximately equal rates of weight gain, nitrogen retentions and serum albumin

levels, there was a higher content of essential amino acids, and of the molar ratio of these to total amino acids in plasma. There were no significant differences in these values between the various wheat diets, suggesting that these two indices might reveal dietary differences in the essential to total amino acid ratio but not in the proportions of the essential amino acids. As lysine was added in increasing amounts to the wheat diets, there was a steady increase in the molar ratio of lysine to total essentials and a fall in that of threonine. These results suggested that the first-limiting amino acid of a moderately deficient diet could be identified from the fasting plasma aminogram. There was enough individual deviation from the mean for the six children, however, to indicate that in a single individual this measure could not be relied upon.

In our experience and that of others (9), approximately 6.4% milk protein calories has been found to be the lower level of adequacy for rapidly growing infants, as measured by rates of growth and serum albumin levels. Consequently, it is not surprising that at 6.0% milk protein calories there was only a slight deviation from the "normal" plasma aminogram of 12.0% protein calories. Most of our evaluations of dietary protein adequacy are carried out at 6.4 to 8.0% protein calories, levels at which significant differences in quality are apparent in the rates of weight gain, nitrogen retention, and serum albumin levels. We have made a study of the fasting plasma aminogram of rapidly growing convalescent malnourished infants and small children receiving one of two milk protein preparations

in this range of protein calories, to serve as a point of reference for studies in which other sources of protein are fed at similar levels. It is hoped that the aminogram will detect in a shorter period of time and with a single blood sample the same differences in quality of dietary protein which normally take many days or weeks to become apparent by the previously outlined methods.

Methods

Fasting plasma was obtained from infants and small children who had been admitted to the British American Hospital for the treatment of severe primary malnutrition at least six weeks previously, who were apparently free of infection, were gaining weight steadily, and had steadily increasing or already normal serum albumin levels. They were all between 4 and 24 months of age and in each case in which it was measured, had demonstrated retentions of at least 20 mg of nitrogen for every gram of weight gained. Plasma was obtained after a minimum of nine days on diets whose only source of protein was a modified cow's milk[1] or calcium caseinate[2]. In both diets 53% of non-protein calories were derived from fat and 47% from carbohydrate. To achieve the desired calorie and protein intakes, cottonseed oil and cane sugar were added to the modified milk and the same plus corn starch to the casein. A "complete" vitamin and mineral mixture (7) was added to the casein diets; appropriate additions were made to the modified milk diets when their originally adequate vitamin and mineral contents had been diluted to achieve the desired percentage of protein calories. Water intake was mildly restricted to assure complete consumption of the diets, which were recalculated daily on the basis of body weight.

Plasma samples were stored at -20° C. until analysis, when their free amino acids were measured by liquid-column chromatography (10). Although tryptophan was usually determined separately, it was not included in the calculations. Cystine, because its level varies independently of dietary protein adequacy, was also excluded from the calculations, thus leaving eight "essential" and "semiessential" amino acids to be taken into account: leucine, isoleucine, valine, lysine, threonine, tyrosine, phenylalanine and methionine. Of the "non-essential" amino acids measured, taurine, because we have found it very susceptible to non-nutritional influences, and aspartic and glutamic acids, because of their pronounced susceptibility to change with frozen storage, were excluded from our calculations, which included alanine, arginine, asparagine, glutamine, glycine, histidine, ornithine, proline and serine. Because of incomplete separation in most cases, the values for serine, asparagine and glutamine have been combined.

Results

Satisfactory fasting plasma samples were obtained from 39 infants and children who for a minimum of nine days had been receiving diets in which modified cow's milk or casein was the sole source of protein. Total caloric intake was 100 to 150 Kcal/kg/ body weight/day and protein intake 1.7 to 3.0 g/kg/day. Protein calories ranged from 6.4 to 8.0% of the total, depending on the respective intakes.

For purposes of comparison in Table 1 the results of the aminograms have been divided according to the source of protein,

modified cow's milk or casein, and according to the level of protein calories, 6.4 - 6.9% and 8.0%. The values for total amino acids were moderately higher for casein at both levels, and this was true for some individual essential as well as non-essential amino acids, probably the result of more complete intestinal absorption: the apparent absorption of nitrogen from casein in our metabolic unit is consistently between 85 and 90% of intake, while that from modified milk is more variable, usually between 75 and 85% of intake. There were very few differences which could be attributed to the level of protein calories: at 8.0% the combined values for serine, glutamine and asparagine were slightly lower and the individual values for lysine and histidine were higher than at 6.4% - 6.9% protein calories.

In figure 1 the mean molar concentration of each amino acid in the 39 samples has been plotted along with one standard deviation above and below the mean. In the case of serine + glutamine + asparagine, only one half the combined molar concentration has been plotted.

Figure 2 has been adapted from Snyderman et al (4), showing their mean values (in mg/100 ml), along with one standard deviation above and below the mean, for plasma free amino acids of infants receiving 12.0% milk protein calories. Our own mean values (plus or minus one S.D.) for those individual amino acids which are included in this report are shown by vertical bars in the same figure. Despite the fact that milk protein provided only 6.4 to 8.0% of calories, most of our means were within their normal range. Our

mean for tyrosine fell just below the lower limit of their normal range. In their studies this was the essential amino acid most affected by lowering the protein intake. Our means for glycine and arginine were clearly above the upper limit of their normal range. In their studies glycine was the non-essential amino acid most affected by lowering the protein intake. Arginine was also elevated but not to the same relative degree as in our studies. If we had plotted the individual values for serine in which the separation was quite clear, they would have shown an elevation similar to that of Snyderman et al.

In Table II we have listed for the four groups the 8 individual essential amino acids as a molar fraction of total essential amino acids (excluding typtophan and cystine) and these in turn as a fraction of total plasma free amino acids. In the last column the values for all 39 samples have been combined and are listed along with their standard deviations. It is apparent that with the possible exception of lysine, neither the source of milk protein nor the level of protein calories affected these ratios appreciably.

A few samples of plasma from infants receiving less than 6.4 or more than 8.0% milk protein calories were available. When the modified milk provided only 5.3% protein calories, the values for serine (+ glutamine + asparagine) and alanine were above, and those for threonine were below our normal range. When casein provided 5.3 to 6.0% protein calories, only alanine was just above our upper limit, the remaining amino acids were within one S. D. of our mean.

At 12.0% protein calories from casein, glycine and arginine were just below our lower limit and threonine just above our upper limit, in accord with the values of Snyderman et al.

Discussion

Snyderman et al (4) already indicated the presence of an effective homeostatic mechanism which maintains the concentration of free amino acids in plasma within a relatively narrow range, presumably ideal for protein synthesis in cells. In experimental animals it has been shown that the marked fall in the concentration of some individual amino acids which is brought about by dietary amino acid imbalances is possibly responsible for the decrease in appetite which these produce (11). No such mechanism has thus far been identified in human beings, although it is quite possible that it exists. If so, it must not be as sensitive as in the rat: the adult or immature human being continues to consume for a long time diets which are notoriously poor in their content of an essential amino acid and which result in severe depression of its concentration in plasma, the best example being the pellagrogenic diet (12).

The present studies, although revealing some alterations which could be attributed to differences in the source or level of milk protein in the diet, generally support the existence of a very efficient homeostatic mechanism. They do suggest that the generally more complete absorption of nitrogenous compounds from our casein diets resulted in moderately higher concentrations of total amino acids in fasting plasma. Because most of the fat and carbohydrate

in the casein diets was of a different origin and nature than in the modified milk diets, the differences in absorption cannot be attributed to protein quality. As a matter of fact, the total fasting plasma free amino acid levels after a number of weeks on diets with wheat flour as the only source of protein were more like those of our casein diets than the modified milk diets, probably as a result of the very efficient nitrogen absorption (8).

The molar ratio of essential to total amino acids in plasma was not affected by the source or the level of milk protein calories in this study, being consistently close to 0.300. In the wheat flour studies (8), this ratio remained close to 0.240 despite additions of lysine which significantly enhanced the biological value of the wheat protein, bringing it very near to that of casein. In both the modified milk and casein, nearly 50% of the total nitrogen is in the form of essential amino acids, while in white wheat flour they represent only 34% of the total. These results suggest that at similar levels of intake in healthy infants, the ratio of essential to total amino acids in plasma is to a great extent a function of this same ratio in the dietary protein.

As already indicated, the concentration of many individual amino acids in plasma was slightly higher on the casein diets, particularly so for those present in higher concentration: serine + glutamine + asparagine, alanine, glycine, proline and valine. It was also higher for leucine and isoleucine, almost certainly not the result of differences in quality. Within the range studied, 6.4 to 8.0% protein calories, the level of milk protein in the diet

had relatively little effect on individual concentrations, with the possible exception of the values for lysine and histidine, with which they were positively correlated. At 12.0% protein calories, in a few samples of our own and in those of others (4), the concentrations of the non-essentials glycine and arginine were lower and that of the essential threonine was higher than for the 6.4 - 8.0% diets. At lower levels of intake, particularly with modified milk as the source of protein, there was apparent an increase in the concentration of some non-essentials and a decrease in some of the essentials, as found by Snyderman et al (4).

When individual essential amino acids were expressed as a molar fraction of total essentials, the only one moderately affected by the concentration (but not the source) of protein in the diet was lysine, as expected from its absolute concentration. In our studies with wheat flour the molar fraction of lysine was sensitive to its level in the diet (8). From the present studies one cannot postulate any difference in protein quality between casein and modified milk. In whole cow's milk protein it has been shown that non-essential nitrogen is the first-limiting factor for the human infant (13). This may well be true of casein as well.

Summary

Within a relatively narrow range of dietary protein calories, 6.4 to 8.0% of the total, neither the source of milk protein nor its level had a significant effect on fasting plasma total free amino acids, the ratio of essential to total amino acids, or the molar

fraction of individual essential amino acids of convalescent malnourished infants. This level of milk protein calories was presumably adequate for maximal growth by other parameters. Minor differences in total amino acids were probably the result of differences in the efficiency of intestinal absorption. The molar fraction for lysine was suggestively affected by its level in the diet.

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FOOTNOTES

1. Similac with Iron, kindly supplied by Ross Laboratories, Columbus, Ohio.

2. Casec, kindly supplied by Mead Johnson International, Evansville, Indiana.

TABLE I

Plasma free amino acids (in μM/ml) of 39 convalescent malnourished infants receiving one of two milk protein diets at 6.4-6.9 or 8.0% protein calories and growing satisfactorily. Tryptophan, cystine, taurine, aspartic acid and glutamic acid are not included.

% PROTEIN CALORIES	8.0%		6.4-6.9%	
Source of Protein	Mod. Milk	Casein	Mod. Milk	Casein
Number of Samples	(4)	(18)	(5)	(12)
	μM/ml of plasma			
SER + GLU + ASP	0.580	0.666	0.626	0.710
ALA	0.250	0.265	0.252	0.272
GLY	0.231	0.263	0.205	0.252
PRO	0.168	0.215	0.196	0.208
VAL	0.151	0.203	0.164	0.203
THR	0.109	0.108	0.111	0.124
LYS	0.132	0.112	0.078	0.096
ARG	0.095	0.098	0.111	0.105
LEU	0.076	0.098	0.090	0.095
HIS	0.082	0.069	0.041	0.058
ORN	0.057	0.055	0.070	0.062
TYR	0.056	0.055	0.047	0.065
ILEU	0.043	0.055	0.049	0.059
PHE	0.050	0.045	0.050	0.050
MET	0.019	0.024	0.022	0.034
TOTAL	2.093	2.331	2.112	2.393

TABLE II

Eight individual essential amino acids as a molar fraction of their combined value (Ind./EAA) and this as a fraction of total plasma free amino acids (EAA/TAA) in 39 convalescent malnourished infants receiving milk protein at 6.4 to 8.0% of total calories.

% PROTEIN CALORIES	8.0%		6.4-6.9%		6.4-8.0%
Source of Protein	Mod.Milk	Casein	Mod.Milk	Casein	Total ± S.D.
Number of Samples	(4)	(18)	(5)	(12)	(39)
	μM Individual AA/μM Total Essentials				
VAL	0.237	0.290	0.268	0.280	0.283 ± 0.034
THR	0.171	0.154	0.182	0.171	0.165 ± 0.038
LYS	0.208	0.160	0.128	0.132	0.157 ± 0.041
LEU	0.119	0.140	0.147	0.131	0.139 ± 0.018
TYR	0.088	0.079	0.077	0.090	0.080 ± 0.019
ILEU	0.068	0.079	0.080	0.081	0.079 ± 0.012
PHE	0.079	0.064	0.081	0.069	0.070 ± 0.013
MET	0.030	0.034	0.036	0.047	0.032 ± 0.006
EAA/TAA	0.303	0.297	0.286	0.299	0.296
S.D.	0.011	0.026	0.042	0.036	0.024

FIGURE 1

Fasting plasma free amino acids (in μM/ml) of 39 infants receiving diets in which milk protein provided 6.4 to 8.0% of total calories and which were adequate in all other respects to support normal growth over long periods of time. Closed circles represent means, bars one standard deviation above and below the mean.

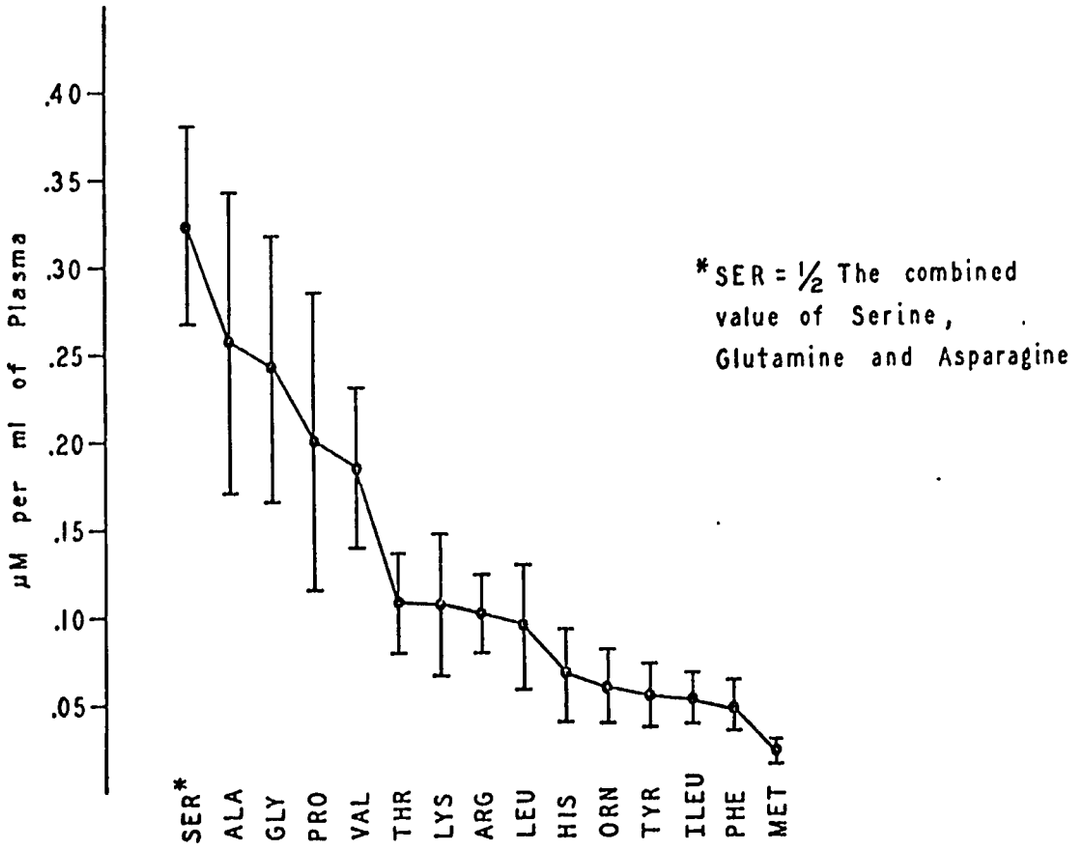
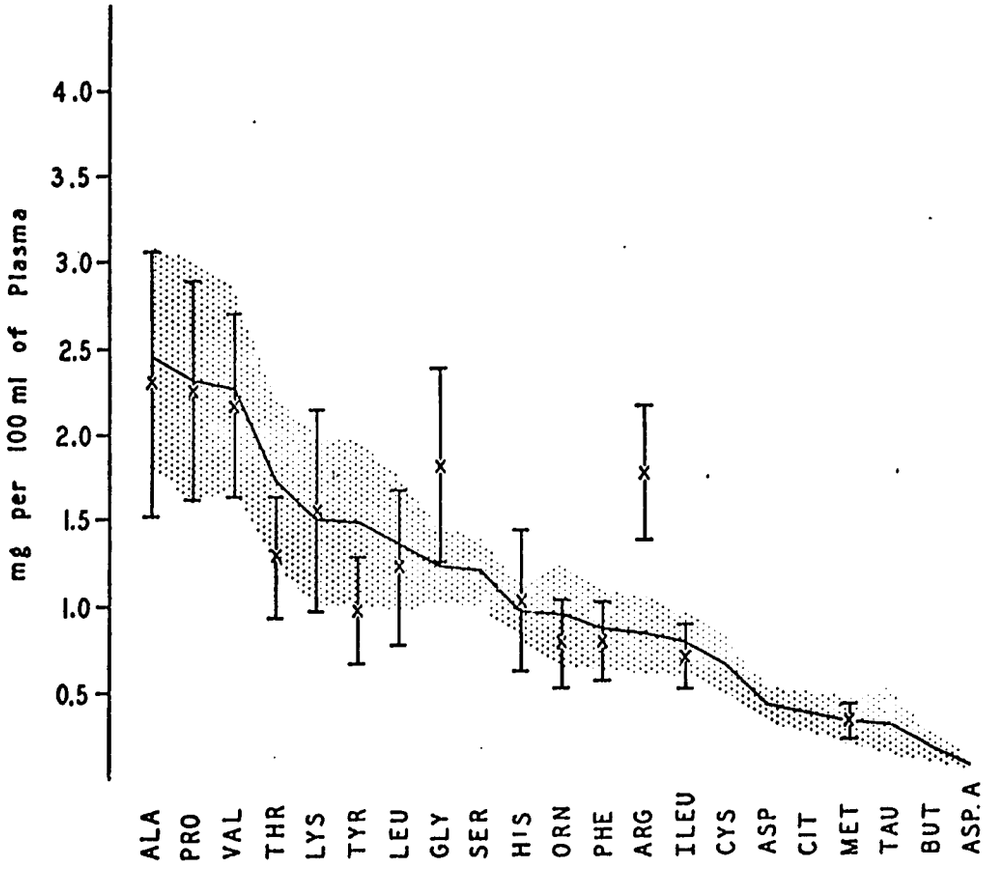


FIGURE 2

Fasting plasma free amino acids (in mg/100 ml) of 39 infants receiving 6.4 to 8.0% milk protein calories. Bars represent one S. D. above and below the mean (X). The continuous line represents the mean and the shaded area one S. D. above and below the mean for infants receiving 12.0% milk protein calories, as reported by Snyderman et al (4).