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SUBJECT:

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Critical Evaluation of New (or Enriched) Protein Sources (for the Prevention of Malnutrition)

Post-prandial Plasma Free Methionine as an Indicator of Dietary Methionine Adequacy in the Human Infant.

ABSTRACT

Fasting plasma free methionine (met) levels were not different in infants consuming met-deficient and met-adequate diets. We have now measured plasma free amino acids (aa) just before and one, two, three and four hours after consumption of a single meal which was either limiting in met, limiting in another aa (lysine or threonine), or probably not limiting in any aa. In the first group post-prandial plasma met was almost invariably below fasting, particularly at three and four hours. In the second group post-prandial met was almost invariably above fasting, while in the third group (balanced diets) it was generally close to the fasting level, but with wider variation. When plasma met is expressed as its molar fraction of total essential and semi-essential aa, it discriminates more sharply between the different diets, notably at four hours.

Indexing Key Words:

plasma methionine
dietary methionine

Most vegetable proteins, when compared to animal proteins or to an "ideal" amino acid mixture, are deficient in one or more of the essential amino acids. Nevertheless, for most of the human race they are the major source of protein in the diet. Even in affluent societies, for both economic and health reasons, they are again assuming some of the prominence they had lost to "milk, meat, and eggs". Fortification with the deficient amino acids, particularly lysine and methionine, to enhance their nutritional value is now possible at a realistic cost. It is of considerable practical importance to determine in the human beings for whom they are intended which amino acids are limiting in a particular protein or mixture, both before and after fortification. On the basis of amino acid composition and of studies in laboratory animals it is possible to make fairly accurate estimates of the degree to which a particular amino acid is limiting, but species differences, both as to requirement and as to digestibility, still make it necessary to confirm or adjust such estimates in humans. The effect of supplementation on nitrogen balance at critical levels of intake has been used effectively in adults and in children, but this technique is established in relatively few institutions and is expensive and time consuming.

In 1961 Longenecker and Hause postulated that postprandial plasma free amino acid levels could be used to identify the limiting amino acids in a diet and indeed to determine their requirement in a fairly rapid manner (1). Young

et al have used the technique to estimate the requirements of tryptophan (2) and of valine and lysine (3) in young adults. In a previous publication (4) we indicated that in infants and young children the fasting or post-prandial levels of lysine did indicate when this amino acid was limiting. Fasting levels of methionine, however, did not effectively indicate when it was limiting. In our hands the nitrogen balance technique has not always indicated when methionine is limiting, there often being no difference when it was presumed to be deficient and when supplementary methionine was added to the diet. In some situations deficiency of methionine (and cystine) has only become evident after prolonged feeding, when serum albumin levels fell or a fatty liver developed.

We are now reporting the post-prandial levels of plasma free amino acids of infants receiving diets deficient in methionine (and total sulfur-containing amino acids), diets clearly deficient in another amino acid (lysine), or diets in which no amino acid was deficient or where the deficient amino acid was not clearly evident. Plasma level of methionine is expressed as its actual molar concentration, as the molar fraction of the total essential amino acids, and as a percentage of the fasting level.

METHODS

The studies were carried out in infants who were well along in their convalescence from malnutrition, whose body weights were already appropriate to length, serum proteins were at normal levels, and clinical condition was excellent. Calorie intakes were adequate to maintain steady gain in weight and protein provided 6.4 to 8.0% of calories, a level critical enough to detect deficiencies in quality of the dietary protein. Non-protein calories were almost evenly divided between fat (mostly cottonseed oil) and carbohydrate (mostly sucrose). Complete vitamin and mineral mixtures were given at all times. Plasma amino acids were determined after an eight-hour fast, before and after the first feeding in the morning, after a number of days on a particular diet or on the day when the protein source was changed. In this last case the fasting levels were to a degree a reflection of the previous diet and the post-prandial levels measured the effect of the new diet. These were determined, one, two, three and in many cases, four hours post-prandially.

Plasma free amino acids were determined by ion exchange column chromatography (5), with the exception of tryptophan, which was determined fluorometrically (6). The molar ratio of a particular essential amino acid is its fraction of the sum of the concentrations of valine, leucine, isoleucine, lysine, threonine, phenylalanine, tyrosine, methionine, cystine and tryptophan. This form of expression tends to correct for differences in concentration that might be due

to changes in the hydration of the patient, or of the plasma sample during storage and processing. It also accentuates any trend, up or down, that might be displayed by one amino acid in contrast to the remaining ones. Post-prandial values are expressed as a percentage increase or decrease from the fasting molar ratio for that particular amino acid, in this case methionine.

A variety of protein sources were used in these studies. The milk used was modified cow's milk[1]. No individual amino acid is first-limiting in these formulas, nor is it, for the human infant, in casein[2]. In these very high quality proteins total nitrogen has been shown to be first-limiting (7). In the soy protein isolate which we used[3] methionine is clearly first-limiting by analysis and in rat studies, but we have had difficulty in demonstrating a supplementary effect by the N balance technique, probably because of its excellent digestibility and utilization. Mx-45 is a mixture of 62.9% whole groat oat flour and 35% defatted soy flour[4]: methionine is first-limiting by calculation but we found excellent nutritional value, without amino acid supplementation (8). WL is a high protein wheat flour[5] enriched with enough lysine to make threonine first-limiting (9). Mx-37 is a mixture of 66% sweet whey solids and 33% full fat soy flour: it has a very high nutritional value and because of its very high lactalbumin content is probably not limiting in any amino acid. Mx-44 is a mixture of 62.9% whole wheat flour and 35% defatted soy flour[4]:

its utilization is probably limited more by digestibility than by any amino acid (8).

RESULTS

In Table 1 we have listed the age of the infants, their diet of the previous day, the protein source of the meal given immediately after the fasting blood specimen was obtained, the fasting plasma free methionine in micromoles/ml and as the molar fraction of total essential amino acids, and the plasma methionine 1, 2, 3 and 4 hours post-prandially as the percentage change of its molar ratio from the fasting level. It is apparent that the molar ratio is less variable than the concentration. The concentration of all the essential amino acids is affected by the ratio of essential to non-essential nitrogen in the dietary protein and by the adequacy of dietary protein relative to total calories.

The first seven studies involved a "test meal" that was clearly limiting in methionine, even though this was seldom apparent by the N balance technique. In 25 of the 27 post-prandial samples, the molar ratio of methionine was lower than the fasting value. The only exceptions were the 2 and 3 hour samples of the infant with the lowest fasting value, who had been receiving the methionine-deficient soy protein for a number of days: the 4 hour value, however, was much lower than the fasting level, as were all others at this time.

The next 8 studies (#8 through #15) involved a "test meal" that was clearly deficient in an essential amino acid other

than methionine: for the first seven it was lysine and for the remaining one, threonine. At one hour 5 of the 8 methionine values were above fasting, at two hours and again at three hours 7 of 8 were above fasting, and at four hours all 4 were above the fasting level.

The last five studies (#16 through #20) involved a "test meal" that was probably not limiting in any amino acid. Here the results were more variable during the first two hours, but at the third hour, all five values were above or very close to the fasting level. Unfortunately, no four-hour specimens were obtained in this group.

In Figure 1 we have plotted the means ±2 standard errors of the mean at each hour for each of the three groups. At one, two and three hours the differences between the methionine-deficient group and the lysine (or threonine)-deficient group are significant but some of the individual values overlap (Table 1). At four hours the difference is highly significant, with no overlap. Although mean values for the third group lie between the other two, they are not significantly different at one, two and three hours.

DISCUSSION

The post-prandial plasma level of free methionine when expressed as the percentage change from fasting of its molar fraction of total essential amino acids, has been found to distinguish, in infants, between diets that are limiting in methionine and diets that are limiting in another essential

amino acid, or in no individual amino acid. When methionine is limiting, its plasma level, relative to the other essentials, is already down one to three hours post-prandially but particularly so at four hours. When methionine is not limiting, or is in apparent surplus because of another amino acid deficiency, its plasma level tends to rise above the fasting level or to remain very close to it, particularly three and four hours post-prandially.

Dietary deficiency of methionine is of importance in diets based on soy protein or other legumes. In our experience this has proven particularly difficult to quantitate by the nitrogen balance technique, as methionine supplementation has not always produced the expected increases in N retention or rates of weight gain. The fall in serum albumin which does occur after along enough period of time on a methionine-deficient diet is not specific enough and can be associated with an increase in liver fat. Plasma amino acid determinations, before and after a single meal, promise to be of assistance in identifying methionine deficiency. Studies are now under way to determine if methionine supplementation of deficient proteins will prevent the expected fall of its plasma level post-prandially.

Our method of expressing plasma levels of methionine, as its molar fraction of the essentials, requires a complete "aminogram", representing considerable time and effort but does increase the sensitivity of discrimination between diets.

In the methionine-deficient diets, if the plasma levels of free methionine in μM/ml are analyzed instead, 20 of the 27 post-prandial values (4/7 at one hour, 4/7 at two hours, 6/7 at three hours, 6/6 at four hours) are below, one is equal, and six are above the fasting level. In the diets deficient in another amino acid 26 of 28 post-prandial values (7/8 at one hour, 7/8 at two hours, 8/8 at three hours, 4/4 at four hours) are above, one is equal, and only one is below the fasting level. In the diets with no amino acid deficiency 10 of 15 post-prandial values (3/5 at one hour, 3/5 at two hours, 4/5 at three hours) are above, one is equal, and four are below the fasting level.

Although the loss of discrimination for methionine-deficient diets seem to be balanced by a gain for those deficient in another amino acid, this is in part due to the tendency of all essential amino acids to rise post-prandially. Whereas by the molar ratio method none of the diets would have been misclassified as to its methionine adequacy, one of the "balanced" diets might have been classified as "deficient" if molar concentration alone had been used. At four hours, however, there was no room for doubt in the samples obtained. It is obvious that further experience with this sample will tell us if the molar concentration of methionine alone will be adequate. This would, of course, simplify the work required, particularly if the one and two hour samples are eliminated altogether.

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FOOTNOTES

1 Either Similac with Iron (Ross Laboratories, Columbus, Ohio) or Lactogeno (Nestle, Peru).

2 Casec, Mead Johnson, Evansville, Indiana.

3 Supro 61, Ralston Purina, St. Louis, Missouri.

4 Quaker Colombia, Cali, Colombia.

5 Beevo, Pillsbury, Minnespolis, Minnesota.

Figure 1 - Post-prandial changes in plasma free methionine (as its molar fraction of the essential amino acids - EAA) after meals which were limiting in methionine (closed circles, solid line), limiting in another aa (closed squares, broken line), or not limiting in any aa (triangles, solid line). Vertical bars are ±2 SEM.

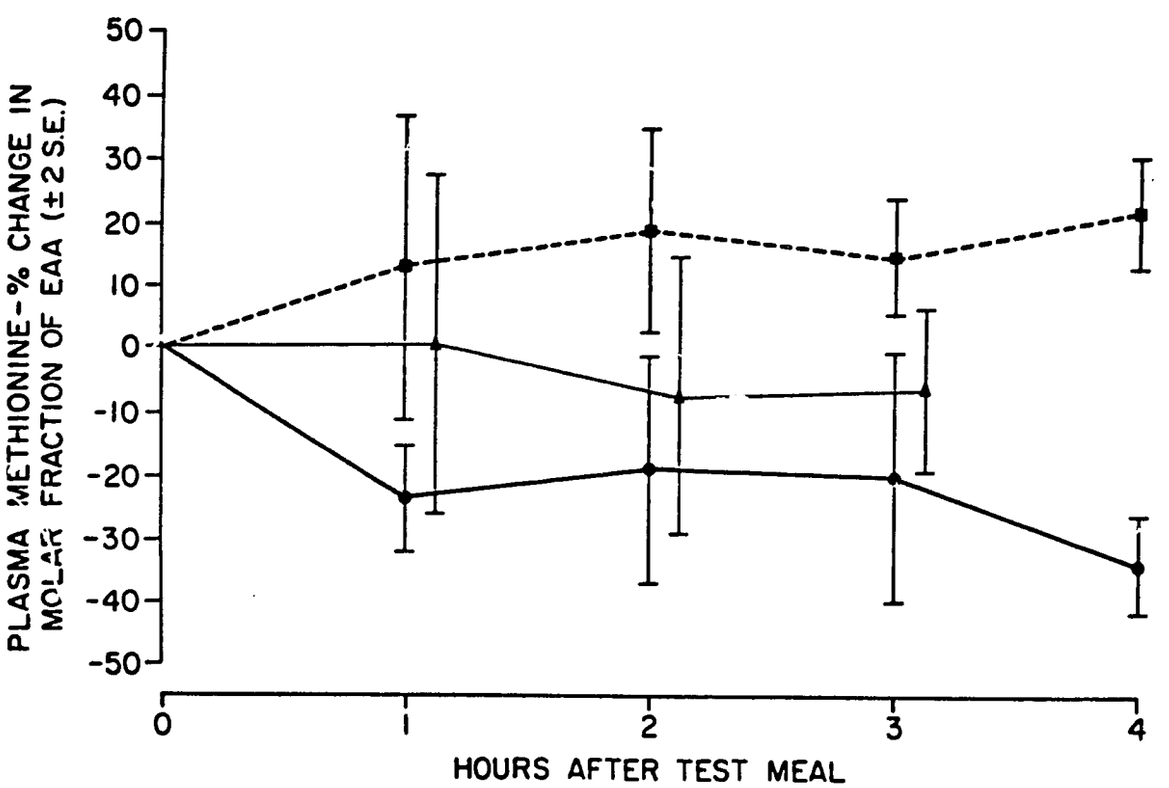

Table 1

Fasting plasma methionine in μM/ml and as the molar ratio of total essential amino acids; post-prandial levels as % change from fasting.

Study #	Case #	Age Mos.	Daily Diet: Kcal /kg	Daily Diet: % prot.	Daily Diet: Prev. day	Daily Diet: Study day	Plasma Methionine: Fasting μM/ml	Plasma Methionine: Fasting Ratio	% change from fasting molar ratio: 1 hr	2 hr	3 hr	4 hr
1	288	17	150	6.7	Milk	Soy[1]	0.042	0.039	-38	-44	-13	-28
2	290	18	150	6.7	"	"	0.030	0.033	-30	-21	-21	-39
3	297	8	150	6.7	"	"	0.021	0.029	-28	-38	-45	-28
4	288	17	150	6.7	Soy	"	0.011	0.018	-11	+28	+33	-50
5	290	18	150	6.7	"	"	0.022	0.027	-30	-4	-19	-37
6	297	8	150	6.7	"	"	0.023	0.036	-11	-31	-44	-22
7	198	16	100	8.0	Mx-45[2]	Mx-45	0.026	0.050	-16	-24	-36	
8	274	9	125	6.4	Milk	Wheat	0.023	0.032	+6	+19	+6	+19
9	277	7	125	6.4	"	"	0.009	0.017	+82	+47	+24	+12
10	203	23	125	6.4	Casein	"	0.017	0.021	+29	+5	+29	
11	274	9	125	6.4	Wheat	"	0.018	0.025	+16	-4	+16	+32
12	277	7	125	6.4	"	"	0.012	0.022	-27	+5	+18	+27
13	203	23	125	6.4	"	"	0.016	0.028	-4	+64	+11	
14	188	14	125	6.4	Casein	"	0.019	0.029	-17	+17	-7	
15	204	13	125	6.4	WL[3]	WL	0.022	0.032	+25	+6	+30	
16	192	22	125	6.4	Casein	Casein	0.023	0.032	-12	-19	-3	
17	202	8	125	6.4	Mx-37[4]	"	0.024	0.037	+3	+5	+19	
18	192	22	125	6.4	WL	"	0.020	0.028	+46	+29	+25	
19	204	14	100	8.0	Mx-44[5]	Mx-44	0.017	0.032	+6	-16	0	
20	194	13	100	8.0	"	"	0.027	0.040	-37	-37	-8	

1 Soy = Soy protein isolate
2 Mx-45 = Oat-soy mixture
3 WL = Wheat flour enriched with lysine
4 Mx-37 = Soy-milk whey mixture
5 Mx-44 = Wheat-soy mixture