APPENDIX V

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Postprandial Plasma Free Amino Acid Changes in Preschool Children Consuming Exclusively Rice Protein

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SUBJECT:

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ABSTRACT

Because of the poor digestibility of rice protein, plasma amino acid fluxes after a rice meal might be expected to be less than those seen after a meal of more readily digested protein. This would militate against finding the changes in plasma essential amino acid concentrations indicative of inadequacy of lysine, the first limiting amino acid of rice protein relative to the FAO/WHO provisional scoring pattern. The postprandial changes of plasma free amino acids were analyzed in fifteen children after consuming a meal in which rice protein provided 6% of 20-25 Kcal/kg body weight. The fasting concentrations of total amino acids (TAA), total essential amino acids (TEAA) and their ratio (TEAA/TAA), reflecting 7-14 days rice consumption, were low (TAA=2353±435 μmole/Liter, TEAA=530±59 μmole/Liter, TEAA/TAA=0.230±0.036). There were no statistically significant postprandial changes in TAA, TEAA, TEAA/TAA, or in the concentrations of Lys or Thr. The Lys/TEAA molar ratio was significantly less than the fasting value at three hours (fasting=0.150±0.030, 3 hour=0.129±0.021, $P<0.02$) but not at 4 hours ($0.10>P>0.05$). No significant change of Thr/TEAA occurred. Despite the generally muted plasma amino acid response to rice protein, the changes seen when lysine is the first limiting amino acid in the diet were found to occur in these studies.

Vegetable proteins are characteristically low in one of the essential amino acids, usually methionine or lysine, occasionally threonine. Recent studies have shown that the methionine deficiency of soy protein and the lysine deficiency of wheat protein can be detected by analysis of postprandial changes of plasma free amino acids of individuals consuming marginal intakes of these proteins (1, 2). Rice protein is acknowledged to be low in lysine (the amino acid score for lysine is approximately 60) (3). Rice protein is considerably less digestible than wheat, for example, and is unique among staple grains in that it is virtually devoid of water soluble matrix protein, all protein being contained in protein bodies that are relatively resistant to digestion (4, 5, 6). For these reasons fluxes of plasma free amino acids following a rice-based meal might be expected to be less than those following a meal containing more highly digestible protein. This would militate against finding the changes in plasma essential amino acid concentrations indicative of lysine inadequacy. Postprandial amino acid values obtained during studies on the protein quality and digestibility of rice (6) have been analyzed. They suggest that despite smaller postprandial fluxes of plasma free amino acids the dietary lysine deficiency of rice protein can be detected.

MATERIALS AND METHODS

Blood samples for determination of plasma free amino acids were obtained during studies designed to compare the protein quality of two varieties of rice, IR 32 (7.1% protein, N x 6.25) and IR 480-5-9

(11.4% protein). The study design has been reported in detail (6) and will only be summarized here. Eight Peruvian mestizo male children, ages 11 to 18 months, participated in the studies with the informed consent of their parents[3]. All were well into convalescence, free of infection and gaining weight. Serum albumin concentrations were all >3.6 gm/dl.

Plasma samples were obtained at 8 a.m. after an overnight fast and then 3 and 4 hours after consuming a meal in which 6% of 20-25 Kcal/kg body weight was supplied by rice protein (N x 6.25). In diets based on IR 32, rice provided all protein and 75% of energy. Rice provided all protein and 48% of energy in the IR 480-5-9 diet. Twenty-seven percent of energy was derived from sucrose in the latter. Both diets contained 25% of energy as a blend (80:20) of soybean and cottonseed oil. Order of consumption of the diets during balance studies was alternated and consequently the fasting values for plasma free amino acids reflected either seven or 14 days of exclusive rice protein consumption.

Plasma was separated within 30 minutes of sampling, was sealed in glass ampules and then frozen at -20°C until shipment in dry ice to Baltimore. Plasma free amino acids were determined by liquid column chromatography (7-9) using an internal standard of norleucine. Tryptophan was determined separately by a photofluorometric procedure (10).

Postprandial changes of concentrations (μmole/Liter) of total amino acids, total essential amino acids and of individual essential amino

acids and of individual essential amino acids, as well as changes of calculated molar ratios were analyzed using a "t" test for paired comparisons (11).

RESULTS

The fasting and postprandial values for the concentrations of total amino acids (TAA), total essential amino acids (TEAA), lysine and threonine, and the molar ratios of TEAA/TAA, Lys/TEAA and Thr/TEAA are shown in Table I. Values for threonine have been included since relative to the FAO/WHO provisional scoring pattern (12) it should be the second most limiting amino acid in rice protein. When three-hour and four-hour values were compared with fasting values, no significant changes occurred in TAA, TEAA or any of the individual essential amino acids. The modest increase of TEAA from a fasting value of 530±59 μmole/Liter to the three-hour value of 574±95 μmole/Liter approached statistical significance ($t=2.100$, $0.10>P>0.05$). Although lysine appeared to decrease slightly the change was not significant. Reanalysis comparing the maximal value at either three or four hours with the fasting value also failed to demonstrate significant changes in TAA and TEAA.

The fasting molar ratio of TEAA/TAA was 0.230±0.036; this did not change significantly at either three or four hours. Nor was a significant difference found when the maximal change at either three or four hours was compared with the fasting value. The molar ratio of Lys/TEAA was significantly less than the fasting value at three hours ($P<0.02$), but not at four hours ($0.10>P>0.05$). Using the maximal

change at either three or four hours, this ratio was also significantly below the fasting value ($P<0.01$). The Thr/TEAA molar ratio showed no significant postprandial change regardless of how analyzed.

DISCUSSION

The postprandial plasma amino acid levels after consuming rice are notable for the lack of change of the concentrations of total amino acids and of the total essential amino acids. Both TAA and TEAA characteristically increase after consumption of a meal containing casein. The same has been reported after consumption of wheat protein (2) and soy protein (1). The protein of wheat is nearly all matrix protein. Other staple grains and legumes have a balance of matrix protein and protein contained in protein bodies. In contrast, rice lacks the more readily digested matrix protein (4). The muted plasma amino acid fluxes seen here are most likely the result of slow and uneven digestion and absorption of amino acids from rice protein bodies coupled with peripheral uptake of amino acids from plasma favored by the well digested energy component of the meal consumed (6). If the latter nearly keeps pace with the former, little change in plasma free amino acid concentrations should be seen.

Both fasting and postprandial values for TEAA/TAA were low. Previous studies have suggested that the fasting TEAA/TAA ratio in plasma is largely determined by dietary protein (13). Ratios of about 0.300 are characteristic for infants consuming milk proteins (13, 14) in which non-essential nitrogen is first-limiting (15). The corresponding value during consumption of wheat protein was found to

be about 0.250 (2). The mean value of 0.223 seen in the present study is quite low in our experience. It is likely that the excellent energy digestibility coupled with the poor nitrogen digestibility of rice (6) accentuated the changes in the TEAA/TAA ratio that were expected during 7-14 days consumption of a lower quality vegetable protein.

Fasting plasma lysine concentrations are relatively resistant to change (2). The fasting concentrations after seven to 14 days of rice consumption in this study are, for example, the same as those reported in healthy low birthweight infants consuming human milk (16). Despite the relative flatness of the TEAA and Lys curves, the postprandial change in the Lys/TEAA ratio was indicative of lysine inadequacy. Previous work has shown that this response can be abolished by the addition of lysine to the level of adequacy, even though lysine may remain the amino acid present in lowest concentration relative to the FAO/WHO provisional scoring pattern (2).

FOOTNOTES

[1] This study was supported by Research Grant AM-HD-10111-02 from the National Institutes of Health, U.S.P.H.S. and by Research Contract AID/Ta-C-1286 with the Agency for International Development, United States Department of State.

[2] Address reprint requests to Dr. William C. MacLean, Jr., 615 North Wolfe Street, Baltimore, Maryland 21205.

[3] These studies were approved by the Joint Committee on Human Investigation, The Johns Hopkins Medical Institutions and the corresponding committee of the Instituto de Investigacion Nutricional, Lima, Peru. Informed consent was obtained from the parents of all children participating in the studies.

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Table I

Fasting and postprandial amino acids of children consuming a meal in which rice protein provided 6% of 20 Kcal/kg body weight. Concentrations of total amino acids (TAA), total essential amino acids (TEAA), lysine and threonine are shown along with the molar ratios of TEAA/TAA, Lys/TEAA and Thr/TEAA. Means ± standard deviations are shown

		TAA - µmole/L			TEAA - µmole/L			TEAA/TAA			Lys - µmole/L			Lys/TEAA			Thr - µmole/L			Th
Diet	n	0[1]	3 hr[2]	4 hr[3]	0	3 hr	4 hr	0	3 hr	4 hr	0	3 hr	4 hr	0	3 hr	4 hr	0	3 hr	4 hr	0
IR 32	7	2426 ±500	2360 ±483	2303 ±480	553 ±64	574 ±108	530 ±99	0.234 ±0.040	0.248 ±0.037	0.234 ±0.035	87 ±16	74 ±20	62 ±[illegible]	0.158 ±0.025	0.128 ±0.022	0.118 ±0.020	78 ±29	75 ±33	72 ±31	0.140 ±0.040
IR 480-5-9	8	2290 ±393	2600 ±449	2367 ±272	510 ±50	575 ±90	552 ±36	0.227 ±0.034	0.223 ±0.029	0.235 ±0.018	73 ±14	74 ±16	80 ±13	0.144 ±0.034	0.130 ±0.021	0.145 ±0.020	82 ±19	98 ±52	87 ±32	0.162 ±0.036
Combined	15	2353 ±435	2488 ±465	2337 ±370	530 ±59	574 ±95	542 ±71	0.230 ±0.036	0.235 ±0.034	0.234 ±0.026	79 ±17	74 ±18	72 ±16	0.150 ±0.030	0.129* ±0.021	0.132 ±0.024	80 ±23	87 ±44	80 ±31	0.152 ±0.038

1 Fasting value. Reflects 7-14 days consumption exclusively of rice protein

2 3 hours postprandially

3 4 hours postprandially

* Differs from corresponding fasting value, $P<0.02$

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Diet	n	TAA - µmole/L			TEAA - µmole/L			TEAA/TAA			Lys - µmole/L			Lys/TEAA			Thr - µmole/L			Thr
		0[1]	3 hr[2]	4 hr[3]	0	3 hr	4 hr	0	3 hr	4 hr	0	3 hr	4 hr	0	3 hr	4 hr	0	3 hr	4 hr	0
IR 32	7	2426 ±500	2360 ±483	2303 ±480	553 ±64	574 ±108	530 ±99	0.234 ±0.040	0.248 ±0.037	0.234 ±0.035	87 ±16	74 ±20	62 ±14	0.158 ±0.025	0.128 ±0.022	0.118 ±0.020	78 ±29	75 ±33	72 ±31	0.140 ±0.040
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