See corresponding articles on pages 756 and 761.

Got some amino acids to spare?[1,2]

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INTRODUCTION

Estimating requirements for the nutritionally indispensable amino acids in adult human nutrition is an active area of investigation and is important in assessing and planning diets worldwide. This aspect of protein nutrition has been the focus of considerable research, controversy, and debate since about the mid-1980s, when Young et al (1) concluded that the 1985 FAO/WHO/UNU (2) recommendations for meeting adequate amino acid intakes were far too low. We (1) proposed that more appropriate recommended intakes for most amino acids should be 2–3 times higher than the FAO/WHO/UNU recommendations. Also, since that time, there has been a significant expansion of research focused on tracer-based estimates of adult amino acid requirements, especially by groups in Toronto (3) and Bangalore, India (4), and by our group at the Massachusetts Institute of Technology. Three recent papers in the Journal authored by the Toronto group (3, 5, 6), including 2 in this issue (3, 5), raise many questions about the quantitative, nutritional relations between the sulfur amino acids methionine and cyst(e)ine on the one hand and the aromatic amino acids phenylalanine and tyrosine on the other. These questions require resolution before comprehensive amino acid requirement scoring patterns can be established for evaluating the nutritional value of food proteins in human nutrition.

SULFUR AMINO ACIDS AND CYSTINE SPARING

It is assumed that the requirement for the sulfur amino acids methionine and cystine can be met from methionine alone, although it is more usually achieved through a combination of methionine and cystine. The results of nitrogen balance studies in men and women suggest a methionine requirement of $\approx$13 mg$\cdot$kg$^{-1}\cdot$d^{-1} when no dietary cystine is present (2). Furthermore, [^{13}C]methionine and [^{13}C]cysteine tracer studies carried out at the Massachusetts Institute of Technology suggest that the requirement for methionine and cystine is not met when the methionine intake is $\approx$6 mg$\cdot$kg$^{-1}\cdot$d^{-1} in the presence of an equal or higher cystine intake; sulfur amino acid balance appears to be achieved at a mean methionine intake of $\approx$13 mg$\cdot$kg$^{-1}\cdot$d^{-1} in the absence of cystine (7, 8). Now, the indirect amino acid oxidation (IAAO) paradigm has been applied by the Toronto group (3), who concluded that the mean methionine requirement, in the absence of dietary cystine, is $\approx$13 mg$\cdot$kg$^{-1}\cdot$d^{-1}. It is not known whether overall sulfur amino acid status and function would improve after consumption of 13 mg methionine$\cdot$kg$^{-1}\cdot$d^{-1} together with cysteine, as opposed to without cysteine, but the other study by the Toronto group (5) adds further understanding in this area.

A significant sparing effect of dietary cyst(e)ine on the methionine requirement has long been accepted in human protein nutrition. However, [^{13}C]methionine (8) and [^{13}C]cysteine (7) tracer studies have not shown a measurable sparing of methionine oxidation at methionine intakes of 5–13 mg$\cdot$kg$^{-1}\cdot$d^{-1} and similar cystine intakes. The short-term IAAO study by Di Buono et al (5) in this issue of the Journal, therefore, is instructive. These investigators gave 6 healthy men methionine intakes ranging from 0 to 13.0 mg$\cdot$kg$^{-1}\cdot$d^{-1} together with a constant intake of 21 mg cysteine$\cdot$kg$^{-1}\cdot$d^{-1}. A standard, fully adequate diet was provided for 2 d before the 5–6-h IAAO tracer study with indicator [^{13}C]phenylalanine. The tracer study was conducted while subjects were consuming an L-amino acid–based liquid formula diet as small hourly meals. The results showed that the breakpoint (used to identify the requirement intake) in the response curve depicting the relation between methionine intake and $^{13}CO_2$ excretion occurred at a methionine intake of 4.6 mg$\cdot$kg$^{-1}\cdot$d^{-1}. Although the abstract to this paper (5) reads as though the study also included a series of diets supplying methionine without cystine, this particular phase is described in the other publication by these investigators and appeared to involve a different group of volunteers (3). By comparing the results of their 2 studies, Di Buono et al (5) concluded that the relatively high dietary cysteine intake of 21 mg$\cdot$kg$^{-1}\cdot$d^{-1} reduces the mean methionine requirement in men by $\approx$64%. However, there were many limitations to this comparison:

1) The rate of $^{13}CO_2$ release from the L-[1-^{13}C]phenylalanine tracer at the methionine intakes above the respective breakpoints differed between the 2 studies; it was $\geq$0.5 μmol$\cdot$kg$^{-1}\cdot$h^{-1} in reference 5 and $\leq$0.4 μmol$\cdot$kg$^{-1}\cdot$h^{-1} in reference 3, when methionine was given without cysteine. This is curious because the experimental conditions (including the tracer infusion rates), other than the methionine and cysteine intakes, were otherwise identical.
2) There appears to be no sparing of endogenous methionine utilization by cysteine at the zero or very low methionine intakes when the rates of $^{13}CO_2$ release in these 2 studies are compared.

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3) Even on the assumption that a sparing of methionine was definitely shown, the practical implications of this finding and those findings emerging from the earlier nitrogen balance studies remain unclear. This is largely because the cyst(e)ine intake (21 $mg \cdot kg^{-1} \cdot d^{-1}$) studied, relative to that of methionine, at the so-called requirement or "breakpoint" of 4.5 $mg \cdot kg^{-1} \cdot d^{-1}$ in reference 3 and that used in an earlier nitrogen balance study (2) are not characteristic of typical diets. Thus, the ratio of methionine to cystine (by wt) in most of the major food proteins (eg, rice, corn, soybeans, meats, and fish) ranges from ≈1 to 2; the ratio in human milk, wheat, and oats is relatively low (≈0.6).

Hence, I conclude that there is no definitive evidence indicating that the methionine requirement can be reduced by cysteine to as substantial a degree as that proposed by Di Buono et al (5) when the ratio of methionine to cystine intakes more closely mimics that of typical diets. This conclusion does not challenge the fact that a sparing of methionine oxidation by cystine occurs. The elegant studies by Finkelstein et al (9), which exploit an in vitro enzyme system, indicate that a sparing by cystine is indeed achieved by a reduction in the synthesis of cystathionine, which is related to a decrease in the concentration of cystathionine β-synthase and of *S*-adenosylmethionine—a positive effector of the enzyme. However, we observed no change in plasma cysteine flux when cysteine intakes are altered over a relatively wide range (7, 8). This observation presumably reflects a significant first-pass uptake of dietary cystine in the splanchnic region, possibly in intestinal tissues, and might imply that a relatively high cysteine intake is required to raise liver or tissue cysteine to concentrations that cause a redistribution of homocysteine between competing remethylation and transsulfuration reactions (9). Thus, how the minimum requirement for total sulfur amino acids might be optimized via a suitable combination of methionine and cystine is still uncertain. This might be approached, initially, through 24-h IAAO balance studies in which various methionine intakes are studied with a different but constant series of cystine intakes (4).

AROMATIC AMINO ACIDS AND TYROSINE SPARING

Tyrosine sparing of the phenylalanine requirement is a related problem to which the Toronto group has also contributed a recent study (6), but with a somewhat different design and aim than that of Di Buono et al's (5) cysteine-sparing study. In the absence of a dietary supply of tyrosine, the phenylalanine requirement would be that intake just sufficient to meet the metabolic needs for these 2 aromatic amino acids. Thus, Roberts et al (6) fed their subjects a constant intake of 9 mg phenylalanine $\cdot kg^{-1} \cdot d^{-1}$ over a 12-h period and studied the effects of an increasing tyrosine intake on the oxidation of L-[1-^{13}C]lysine as an indicator of tyrosine and aromatic amino acid adequacy. This phenylalanine intake was considered sufficient on the basis of the results of an earlier study in which L-[1-^{13}C]phenylalanine was measured after the consumption of various phenylalanine intakes concomitantly with a large excess of tyrosine (40 $mg \cdot kg^{-1} \cdot d^{-1}$) (10). In the study by Roberts et al (6), a breakpoint in the relation between lysine oxidation and tyrosine intake occurred at a tyrosine intake of 6 $mg \cdot kg^{-1} \cdot d^{-1}$. However, at this intake, it can be estimated from the data presented on the relation between lysine intake and lysine oxidation that an intake of 9 mg phenylalanine $\cdot kg^{-1} \cdot d^{-1}$ might well have been limiting. If so, then no further improvement in overall amino acid utilization, as reflected by the rate of indicator oxidation, would be expected with a tyrosine intake >6 $mg \cdot kg^{-1} \cdot d^{-1}$ and especially because the ratio of phenylalanine to tyrosine (by wt) in body proteins is greater than unity. If this interpretation of the data is valid, a total aromatic amino acid requirement of 15 mg (9 mg phenylalanine and 6 mg tyrosine)—as proposed by Roberts et al (6)—would be limiting and insufficient to meet the physiologic needs for total aromatic amino acids.

Furthermore, using L-[1-^{13}C]tyrosine as a tracer, Basile-Filho et al (11) observed that after a dietary phenylalanine intake of 18.5 $mg \cdot kg^{-1} \cdot d^{-1}$ (with the phenylalanine and tyrosine tracers the total aromatic amino acid intake was 25.3 $mg \cdot kg^{-1} \cdot d^{-1}$), subjects were in distinctly negative whole-body aromatic amino acid balance, but were at equilibrium at a total aromatic amino acid intake of 42.4 $mg \cdot kg^{-1} \cdot d^{-1}$. Thus, the total aromatic amino acid requirement may be from 25 to 40 $mg \cdot kg^{-1} \cdot d^{-1}$. Additional support for this suggestion comes from the results of an interesting investigation by Kindt and Halvorsen (12) in two 6-y-old children with phenylketonuria and in one child with a tyrosine transaminase defect. The intake of phenylalanine that kept plasma phenylalanine and tyrosine concentrations within a clinically expectable range, while supporting normal growth, in the children with phenylketonuria was ≈20 $mg \cdot kg^{-1} \cdot d^{-1}$. The comparable combination of phenylalanine and tyrosine intakes in the child with a tyrosine transaminase defect was ≈60 $mg \cdot kg^{-1} \cdot d^{-1}$.

Finally, the Toronto group (13) estimated with an IAAO tracer study in 6–9-y-old children with classic phenylketonuria that the mean tyrosine requirement is 16–19 $mg \cdot kg^{-1} \cdot d^{-1}$. If the phenylalanine requirement for these children approximates the mean habitual intake of 24 mg phenylalanine $\cdot kg^{-1} \cdot d^{-1}$ (as established by clinical monitoring) or perhaps a lower intake of 14 $mg \cdot kg^{-1} \cdot d^{-1}$ (as determined with a tracer approach; 14), then the total aromatic amino acid requirement would be in the range of ≈30–45 $mg \cdot kg^{-1} \cdot d^{-1}$. Because the principal utilization of amino acids in young children and adults is for maintenance (2), these growth-tracer genetic studies (12–14) add to the view that a mean aromatic amino acid requirement for maintenance in healthy adults is probably higher than the value derived by Roberts et al (6).

SUMMARY

In summary, tracer studies directed toward improving quantitative estimates of the indispensable amino acids requirements in adults continue to enhance our knowledge of protein nutrition in humans. Studies in adults are particularly important because the results of such investigations help in assessments of the amino acid requirements for other age groups and vice versa. It is clear that significant questions remain. Importantly, it is hoped that valuable studies such as those conducted by the Toronto group (3, 5, 6, 13, 14) will catalyze an expanded interest in research on the use of tracer techniques for estimating amino acid requirements, as well as those of nutrients in general.

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