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CURRENT CONCEPTS ON REQUIREMENTS OF ESSENTIAL AMINO ACIDS

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The requirements of essential (indispensable) amino acids (IAAs) are the amounts that the diet must provide to balance obligatory losses, utilization and oxidations, and to allow non-IAA synthesis, protein repletion, tissue maintenance and growth consistent with long-term good health and bodily functions.

Current international requirement values (1) refer only to the L-α-amino acids that participate in protein synthesis and which the human body cannot produce (ileu, leu, lys, met, phe, thr, trp, val and, in infants, his). They derive from investigations by few groups of scientists in the U.S.A., Japan and Guatemala, which differed in experimental designs, metabolic principles of the methods and evaluation criteria.

Studies by Snyderman, Holt and collaborators (2): Each IAA was studied in 5-8 babies, 6 days-7 months old (median age: 1.5 months). During 2-16 weeks they ate a mixture of 18 L-AAs in ratios similar to human milk. Nitrogen (N) and energy intakes were equivalent to 3 g protein and 125 or 150 kcal/kg/day. The IAA under study was replaced with glycine and reintroduced using between 1 and 4 different amounts of the IAA in an irregular ascending or descending stepwise fashion, for 1-4 weeks. N balance was measured in the last 4 of 6 or 7 days in 2-7 infants with each IAA. The lowest level of intake that allowed adequate weight gain and did not decrease N retention in all children tested for a specific IAA, was suggested as the requirement.

These studies can be criticized because: (a) Several babies were born prematurely and weighed 2.5-2.9 kg at the beginning of the study. Their IAA requirements during catch-up growth might have differed from those of mature infants. (b) Some AAs were studied in very few infants. (c) The excessive N intake affects balance and might influence IAA requirements. (d) The experimental protocol varied between AAs and between children with the same AA (levels of intake, duration of dietary and balance periods, outcome variables, number and ages of infants). (e) Unspecified amounts of cys and tyr in the diet complicate assessment of met and phe requirements, respectively. (f) 7-day periods may be too short to assess weight gain adequately and 2 days of adjustment to a new intake level may be too short for N balance studies.

Studies by Fomon et al. (3): 22 and 13 healthy infants, respectively, ate cow's milk or soy-based formulas, from 8 to 112 days of age. Mean intakes were around 1.65 g milk protein, 1.74 g soy protein and 102 kcal/kg/day (coefficients of variation = 6-9%), plus small amounts of strained fruits and oatmeal or rice cereal. Three-day apparent N balance was measured once in 6 infants and on 6-8 times in 5 others. The average amounts of IAAs eaten by each child who grew adequately and had normal levels of serum albumin were calculated. The highest of the range of values were suggested as the requirements.

The main criticisms of these studies are: (a) The amounts of some -or all-IAAs eaten ad libitum may be in excess of the requirements. (b) Requirements of phe were calculated in presence of unspecified amounts of tyr.

The FAO/WHO/UNU Committees (1) used the lower value suggested for each IAA

by the two groups of investigators. However, Holt and Snyderman's (2g) comments on their own study with leu, and a careful analysis of Fomon's studies (3b, c), suggest that the values for leu, met + cys, phe + tyr, and thr should be reduced by 7, 27, 5 and 11%, respectively.

Studies by Torún, Pineda and collaborators (4-5): Each of 6 IAAs (excluding leu and phe + tyr) was studied in 6 well nourished boys, 1.8-2.5 years old (4). Dietary N derived from 0.3 g cow's milk protein plus a mixture of L-AAs in the same ratios as in 0.9 g milk protein/kg/d. Energy intake was 100 kcal/kg/d. Each IAA was studied at 5 levels of intake, between the equivalents of 0.3 to 1.2 g milk protein/kg/d, replacing it with glycine. Each level was fed for 9 days in an ascending order in 3 children, and descending in other 3; during the last 4 days, "true" N balance (allowing 8 and 16 mg/kg/d for miscellaneous losses and growth retention, respectively), urinary urea/creatinine (u/c) ratio and free AAs in plasma were measured. The lowest level of intake before the concentration of the specific IAA began falling, and before positive N balance began decreasing, and before u/c began increasing in all children, was suggested as the requirement or, rather, the safe level of AA intake. The validity of these values was confirmed when the safe levels of intake (SLI) of cow's milk or soy protein were studied in 10 other boys (4b,5). The requirements (or SLI) of leu and phe + tyr were calculated from the milk and soy studies.

These studies may be criticized because: (a) the amounts of leu and phe + tyr may be in excess of the requirements; (b) the values for lys, met + cys and thr exceeded by 9-12% the AA contents in the SLI of milk or soy protein.

Studies by Nakagawa and collaborators (6): Each IAA was studied in 2-5 boys (thr in 8) 10-12 years old. The diets had 13 or 17 AAs that provided 12 g N/d (equivalent to 1.7-3.6 g protein/kg/d). Energy was 46-84 kcal/kg/d. The requirement value that was calculated for an IAA was fed in subsequent studies of other IAAs. The IAA under study was partly replaced with gly and provided in 3 or 4 levels of non-uniform amounts, in ascending or descending order. Each level was given for 3-5 days without an adaptation period. N balance was measured without allowing for miscellaneous losses nor growth retention. The lowest level of intake that supported a positive N balance in all children (sometimes as low as +3 mg N/kg/d) was divided into the average weight of the children and suggested as the requirement value.

These studies can be criticized because: (a) Several IAAs were studied in only 2 or 3 boys. (b) The N balance results are dubious because of the very high N intakes, sometimes also high or low energy intakes, no time to adjust to each new level of AA intake, wide variations in the specific IAA amounts in two consecutive balance periods, and "positive" apparent N retentions that were lower than integumental losses and growth needs. (c) Composition of the AA mixture was not uniform, did not include all L-AAs in dietary proteins and sometimes had excessive gly or glu. (d) Body weights ranged widely (21-45 kg) without information about the children's nutritional status.

Studies by Rose and collaborators (7): Each IAA was studied in 3-6 "young" men, weighing 53-82 kg. The diets had a mixture of 8 IAAs plus gly and urea. Total N intake was 7 (try, phe, lys) or 10 g/d (equivalent to about 0.55-1.2 g protein/kg/d) and 7% of the N was derived from D-AAs (ileu, met, phe, thr and val as racemic amino acids). Energy intake was 55 kcal/kg/d. The IAA under study was gradually reduced and replaced with gly, and studied at 3-6 different levels of intake. Apparent N balance (without allowance for miscellaneous losses) was measured for 4-14 days (4-9 days in most). The lowest level of intake that supported a positive N balance in all men (sometimes as low as +1 mg/kg/d) was suggested as the minimum requirement.

These studies can be criticized because: (a) the AA composition of the diet, with urea and excess gly, differs from any dietary protein; (b) N intake approximated minimal values in some men; (c) energy intakes were high for sedentary individuals; (d) the lowest "positive" N balance reported in some men may have been negative if integumental N losses were included in the calculations; (e) some IAAs were studied in only 3 or 4 men.

Studies in Women (8-11): One group of investigators (8) studied 5 IAAs, another (9) two (ileu and met ÷ cys) and two groups (10,11) studied lys. They used similar but not identical designs, with women 19-36 years old, weighing 49-75 kg; diets with IAAs in the ratios of egg protein, 4 other AAs and most non-essential N from gly or gly + diammonium citrate (wheat flour and corn meal in one study); N intakes of 6.5-10 g/d, equivalent to about 0.6-1.1 g protein/kg/d; and 28-54 kcal/kg/d. Each IAA was studied in 6-21 women. It was reduced in 2-6 steps and sometimes increased again after N balance became negative. Apparent N balance was measured for 5-10 days. For most IAAs, N equilibrium was defined as urinary plus·fecal excretion between 95 and 105% of intake. The suggested requirement was the lowest level of intake that supported that equilibrium in all (8), most (10,11) or the average of the group of women.

These studies can be criticized for the same reasons as Rose et al's, and also because: (a) energy intake was low in some women, causing weight loss; (b) the values suggested as the requirements were inadequate to support N equilibrium in several women; (c) a N balance of -5% of intake, which is even lower when miscellaneous losses are included in the calculations, underestimated the intakes needed for "true" N equilibrium in many women. Hegsted (12) did regression analysis of the IAA intake data on N balance and suggested values that were 7-27% larger; if a N retention of 0.5 g/day for miscellaneous losses was used, requirements for individual IAAs would increase by 160-493%, but this was not considered in the FAO/WHO/UNU recommendations (1).

INACCURACY OF CURRENT FAO/WHO/UNU ESTIMATES: A critical analysis of all those studies indicated that: (a) the requirements of infants were most probably overestimated for several IAAs; (b) the values for preschool children are safe levels of IAA intakes, rather than minimal requirements; (c) the accuracy of the estimates for school children is dubious; and, (d) the requirements for adults were underestimated by overlooking the obligatory miscellaneous N.losses and using excessive energy intakes in N balance studies.

Based on current estimates (1), the requirements for IAAs (excluding his) are around 43, 32, 22 and 11% of total protein requirements for infants, preschool children, school children and adults, respectively. Although there is no satisfactory metabolic explanation for this apparent large decrease with age, it led to suggest that the importance of the AA composition of dietary proteins decreases with age, such that in the adult only protein digestibility must be considered to assess the protein quality of almost all diets. That suggestion seems to be wrong, as the apparent age-related differences can be largely explained by the methodological problems discussed above.

Young, Bier and collaboratos (12) followed a kinetic approach to explore the requirements for leu, val, lys and thr in adults. Using stable isotope probes, they estimated the daily AA oxidation and compared it with the AA intake needed to balance oxidative losses from the body, such that daily AA intake - AA oxidation = 0. This resulted in estimates of requirements 2-3 times greater than currently accepted values. They also found that leu balance approached equilibrium with intakes at the currently recommended levels, but at the expense of a reduced rate of body protein turnover, and with an increase in N retention -suggesting protein repletion- when dietary leu was augmented (12f) The functional significance of slower protein turnover has not been clearly assessed but it may be linked to the provision of AAs required for functions such as tissue repair, immunologic activity and responses to stress.

Millward and Rivers (13) suggested a model to assess IAA requirements, which emphasizes the diurnal cycling of protein deposition and mobilization, and a regulatory oxidation of AAs that varies with the diet and should, therefore, be defined in "operative" terms. They criticized several aspects of Young et al's kinetic approach and dissented with their estimates, but agreed that current IAA requirement values for adults are too low. They also suggested that the values for infants and preschool children may be higher than the minimal requirements, but closer to the "operative" values that occur on natural diets.

Young et al (14) re-evaluated the IAA requirements for adults based on

obligatory N losses, body protein turnover rates and their own isotopic studies. Their estimates per kg body weight or per total protein requirements, are very similar to those of Torún et al. for preschool children (4,5). They proposed an AA scoring pattern similar to the latter for application from preschool age through adulthood to assess protein quality (14b). This supports the argument that, except for infants under 1 year of age, the AA pattern for preschool children should be used to evaluate the nutritional quality of proteins.

INDISPENSABILITY OF OTHER AMINO ACIDS: There are contradictory studies about the indispensability of histidine for adults (15,16). However, FAO/WHO/UNU (1) indicated that the requirement for infants is 28 mg/kg/d and for adults 8-12 mg/kg/d, and interpolated values for other age groups. The accuracy of those recommendations is difficult to establish because biochemical or clinical indications of his deficiency (e.g., skin lesions, decreased erythropoiesis) appear slowly in adults and might require relatively low N intakes for several weeks due to the organism's ability to synthesize some his and to the high body stores of this AA in hemoglobin and carnosine.

Cysteine and tyrosine have been called "conditionally indispensable" because in addition to reducing the requirement for their precursor IAA (met and phe, respectively), they may be required in the diet when met and phe are absent or under conditions such as metabolic disorders that prevent their synthesis (e.g., homocystinuria, phenylketonuria), liver cirrhosis and in the biochemically immature, preterm infants (16,17). However, their requirements for healthy individuals with normal diets are linked to that of the precursor and expressed as requirements for total sulfur- or aromatic AAs.

Arginine: The excess N generated by the catabolism of AAs is disposed through the urea cycle, with arginine, citrulline and ornithine as intermediates. When there is an overload of the urea cycle due to increased protein catabolism, there may be an increased need for arg to prevent or decrease the ensuing hyperammonemia. In fact, in some cases of oroticaciduria, acute uremia and Reye's syndrome, the administration of large doses of arg can reduce the ammonia levels (15,16).

Studies on patients with gyrate atrophy of the retina and hyperornithinemia suggest that arg may be the only precursor for ornithine (18), and that orn might also be classified as conditionally indispensable (16). However, there is no evidence for the indispensability of arg and orn for normal humans.

Taurine, a decarboxylated 2-amino acid, is one of the most abundant free AAs in the human body. It is not incorporated into proteins and its best known function is the formation of bile acid conjugates. It also seems to play some role in neural, retinal and cardiac functions (16,19). Some, but not all, studies on preterm infants fed with formulas based on casein and whey proteins devoid of tau, or on cow's milk low in tau, showed a decrease in plasma and urine levels of tau. There were no clinical disorders, but it has been argued that those children may be at risk of developing neural or retinal abnormalities (16). Patients on TPN with formulations without tau for several months, also had low plasma concentrations of the AA. This was more so in children, some of whom also had mild electroretinographic abnormalities (19d,e).

Although it is reasonable to recommend the addition of tau to TPN solutions, its indispensability in normal humans has not been demonstrated, probably because: (a) the large tau pool in the body cannot be depleted in short-term studies, (b) a reduction in tau intake also reduces tau losses by changes in bile salt formation from predominantly tau conjugation to predominantly gly conjugation; (c) diets containing animal foods have significant amounts of tau; and, (d) cys in the diet might be a precursor of tau.

CONCLUSIONS: Current recommendations for IAA intakes and AA scoring patterns must be revised and updated. Recent kinetic approaches based on isotopic studies suggest that IAA requirements for adults -and by inference, for school children- are similar to those of preschool children, when expressed per unit of body weight or in relation to total protein requirements.

The question still remains as to whether studies with mixtures of AAs are nutritionally equivalent to whole proteins. Except for some studies in infants

(3) and preschool children (4,5), the results of investigations with AA mixtures have not been validated.

Finally, the dietary needs for conditionally indispensable and certain dispensable AAs must be further clarified, as well as those for AAs that are not incorporated into body proteins but may play important metabolic roles for the normal function and health of humans.

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