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HUMAN AMINO ACID REQUIREMENTS: Can the Controversy Be Resolved?

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BACKGROUND

It was more than a century after Magendie (cited by Munro, 49) first demonstrated the essentiality of protein in the diet that all the constituent amino acids were discovered and their nutritional importance was established. With the isolation and identification of threonine (41, 45), it became possible, for the

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first time, to contemplate experiments to estimate amino acid requirements directly. Having identified 10 amino acids as indispensable for the growth of the rat, Rose and colleagues embarked in 1942 on a series of nitrogen balance studies with adult men. They removed each of these amino acids separately from the diet and found that eight were required to maintain nitrogen equilibrium. These were valine, methionine, threonine, isoleucine, leucine, phenylalanine, lysine, and tryptophan. Removal of histidine, previously shown to be indispensable for the growing rat (55, 57, 58), had no effect on nitrogen balance in adult men. This finding led Rose (56) to conclude that preformed histidine was not necessary for the maintenance of nitrogen equilibrium in adult men. Indeed, the essentiality of histidine for man was not established until much later (30).

Those first qualitative studies were followed by further nitrogen balance experiments to establish the quantitative need for each of these eight indispensable amino acids. The results of those experiments were published in a series of papers and summarized by Rose (56), who sought to determine for each male subject the amount of amino acid that would induce a "distinctly positive balance." Recognizing that he had studied very few subjects (only 3–6 for each amino acid) and that results were highly variable, Rose designated the highest of these individual values as the "tentative minimum requirement" (Table 1). This procedure would necessarily yield a relatively high estimate. Rose then referred to an intake of double this amount as a "safe intake."

Table 1 Estimates of the essential amino acid requirements (mg/d) for the maintenance of nitrogen equilibr in adult men and women

	Estimates from nitrogen balance experiments			FAO/WHO/UNU (1985)	Estimates from tracer studies
	Men[a]	Women[b]	Women[c]		
Threonine	500	305	375 (270–515)	455	975
Valine	800	650	622 (550–705)	650	1300
Methionine	165	180	194 (164–230)	—	—
Methionine + cystine	1100	550	700 (250–1950)	845	845
Isoleucine	700	450	550 (280–1065)	650	—
Leucine	1100	620	727 (560–940)	910	1950–2600
Phenylalanine	300	220	258 (215–310)	—	—
Phenylalanine + tyrosine	1100			910	—
Lysine	800	500	544 (355–835)	780	1950
Histidine	—	—	—	520–780	—
Tryptophan	250	157	168 (146–193)	228	—

[a] Rose values (56).
[b] Original Leverton values (31).
[c] Recalculated by regression with 95% confidence limits (23).
[d] Young, Bier and Pellet (70) assuming a body weight of 65 kg.

Despite the limitations of these classical nitrogen balance experiments, Rose's estimates and those on women that followed (see next section) have remained for the past 30 years the generally accepted standard on which current international estimates of adult amino acid requirements (16; Table 1) are essentially based. Recently, these estimates have been challenged by new studies based on isotopic measurements of amino acid losses through oxidation (68, 69, 74). This new work has led to much higher estimates of requirements (Table 1) and has given rise to a major controversy (see 47). In this review, we examine the basis of the controversy, which involves both methodological and physiological issues. We discuss the assumptions underlying each view and suggest that, given these assumptions, dissimilar estimates are to be expected. Finally, we suggest what new information is needed to resolve the differences. We draw our evidence from both human and animal studies. We focus exclusively on the requirements of adults, although assessment of the needs of infants and children involves some of the same issues.

REQUIREMENTS BY NITROGEN BALANCE

Following Rose's seminal work a more extensive series of experiments, using similar methods, was undertaken on women by Leverton, Jones, Reynolds, Swenseid, and their colleagues and reviewed by Leverton (31). In these experiments, which appear to have been well conducted (27, 32–36, 54, 60) and which involved many more subjects than Rose's studies, the aim was to identify the amount of each indispensable amino acid that allowed nitrogen equilibrium, defined as the zone in which the difference between intake and excretion did not exceed ±5% of the intake. The estimates so derived are given in Table 2. They are substantially lower than Rose's estimates for men, but this discrepancy is probably due less to a real difference between the sexes than to dissimilarities in procedure and in particular the different way of assessing requirement. Because the way that adequacy was defined in these experiments meant that the subjects did not actually have to reach nitrogen equilibrium, Hegsted (23) later reanalyzed these data, calculating the semilogarithmic regression of nitrogen retention on amino acid intake. His estimates are somewhat higher than the original ones, but in most cases not appreciably so; his analysis clearly demonstrated the wide confidence limits for these estimates (Table 1). Hegsted also used the regressions to estimate the intake needed to maintain a positive balance of 0.5 g N d^{-1}, which he suggested might be desirable. These intakes were 1.6–4.9 times those needed for nitrogen equilibrium, which illustrates the sensitivity of "requirements" to the level of nitrogen retention deemed adequate.

Rose's original experiments had various weaknesses, some of which have already been pointed out by Young, Bier & Pellett (70) and by Young (68). These include the very small number of subjects, the use of racemic mixtures

Table 2 The regression of nitrogen retention (g d^{-1}) on $\log_{10}$ amino acid intake (mg d^{-1}) calculated by Hegsted (23) from the data on adult women (references shown below) and the effect on the estimate of requirement of allowing for unmeasured nitrogen losses of 0.30 g d^{-1}

Amino acid	Regression coefficient	Requirement for zero balance (mg d^{-1})	Requirement for an apparent positive balance of 0.30 g N d^{-1} (mg d^{-1})
Threonine (34)	0.75	375	942
Valine (33)	2.60	622	811
Methionine (54)	1.76	194	287
Methionine + cystine (54)	0.72	700	1827
Isoleucine (60)	1.02	550	1083
Leucine (32)	0.89	727	1580
Phenylalanine (35)	1.46	258	414
Lysine (27)	1.06	544	1044
Tryptophan (36)	2.47	168	222

of amino acids with uncertainty as to the fate of the D-isomers, rather unstructured dietary sequences, and excessive energy intakes. None of these criticisms applies to the later studies with women, reviewed by Leverton (31). However, like Rose, Leverton and colleagues did not consider histidine an indispensable amino acid and did not include it in their mixtures. What effect progressive depletion of body histidine reserves may have had on the utilization of other amino acids is unclear. A more important problem, common to all those nitrogen balance studies and which may have given rise to serious underestimation of amino acid needs, is the accuracy of the nitrogen balance method itself.

Errors in Nitrogen Balance Experiments

It has long been recognized that nitrogen balances tend to overestimate true rates of body nitrogen retention. The likely magnitude of error depends on the exact procedures used. The nitrogen balance experiments by Rose, Leverton, and their colleagues were based solely on urinary and fecal losses. Nitrogen is also lost from the external epithelia and via secretions. Apart from the omission of such losses, nitrogen retention tends to be overestimated because of measurement errors (24). Nitrogen intake is often overestimated as a result of spillage and incomplete recovery of uneaten food, and excreta may be incompletely recovered. Moreover, losses of nitrogen as gaseous ammonia are rarely measured. A combination of these errors leads investigators to overestimate retention (10).

Calloway et al (3) made comprehensive measurements of dermal and other nitrogen losses in adult subjects. Dermal losses increased greatly with exer-

cise-induced sweating and with protein intake. Calloway et al concluded that the total error in nitrogen balance experiments in which these losses are disregarded could be ~ 0.5 g/day, a value essentially the same as 8 mg kg^{-1} d^{-1} (16, annex 6). Although unmeasured losses of this magnitude may well be incurred in typical nitrogen balance experiments with normally active subjects given conventional diets, this adjustment may be more than is appropriate to the experiments reviewed by Leverton (31). For sedentary subjects with nitrogen intakes similar to those used by Leverton and others, Calloway et al (3) estimated the sum of dermal losses (0.15–0.19 g N d^{-1}) and those which they termed unavoidable and fairly constant losses (skin, hair, nails, toothbrushing, etc, of 0.12 g N d^{-1}) at ~ 0.30 g N d^{-1}. Using the regressions that Hegsted (23) calculated from the original balance data, we have estimated the amino acid intake required for an apparent positive balance of 0.3 g N d^{-1}, i.e. probable true nitrogen equilibrium. These values are given in Table 2.

In addition to these recognized errors, the observation that subjects apparently in substantial positive nitrogen balance and with adequate energy intakes nevertheless fail to gain weight has raised the question of whether elemental N can be lost from the body (7). No persuasive positive evidence of such losses has been produced, although the technical difficulty of making the necessary measurement has thus far precluded a definitive answer (15, 38). This question extends beyond the scope of this review.

From this discussion it is clear that estimates of amino acid requirements from nitrogen balance are sensitive to small differences in apparent balance and have wide confidence limits. The technical difficulties of establishing the amino acid intakes that allow true nitrogen equilibrium to be maintained in adapted subjects have never been fully overcome, and the estimates of requirements obtained by this approach are likely to fall below true values.

Does Nitrogen Equilibrium Necessarily Imply Amino Acid Equilibrium?

In the nitrogen balance approach nitrogen equilibrium is taken to imply amino acid equilibrium. This is known to be untrue for histidine (leading Rose to classify it as dispensable). Histidine is unusual in that substantial quantities exist in the form of tissue peptides, carnosine (β-alanyl histidine), anserine (β-alanyl-1-methyl histidine), and balenine (β-alanyl-3-methyl histidine). Of these, only carnosine serves as a labile store of histidine, but (as demonstrated in experimental animals) it may be substantially depleted during dietary histidine deficiency, allowing tissue protein replacement to proceed for a considerable time (5). In addition to carnosine depletion, dietary histidine deficiency is accompanied by the depletion of hemoglobin (11), a protein rich in histidine. The release of histidine from these two sources allows experimental animals

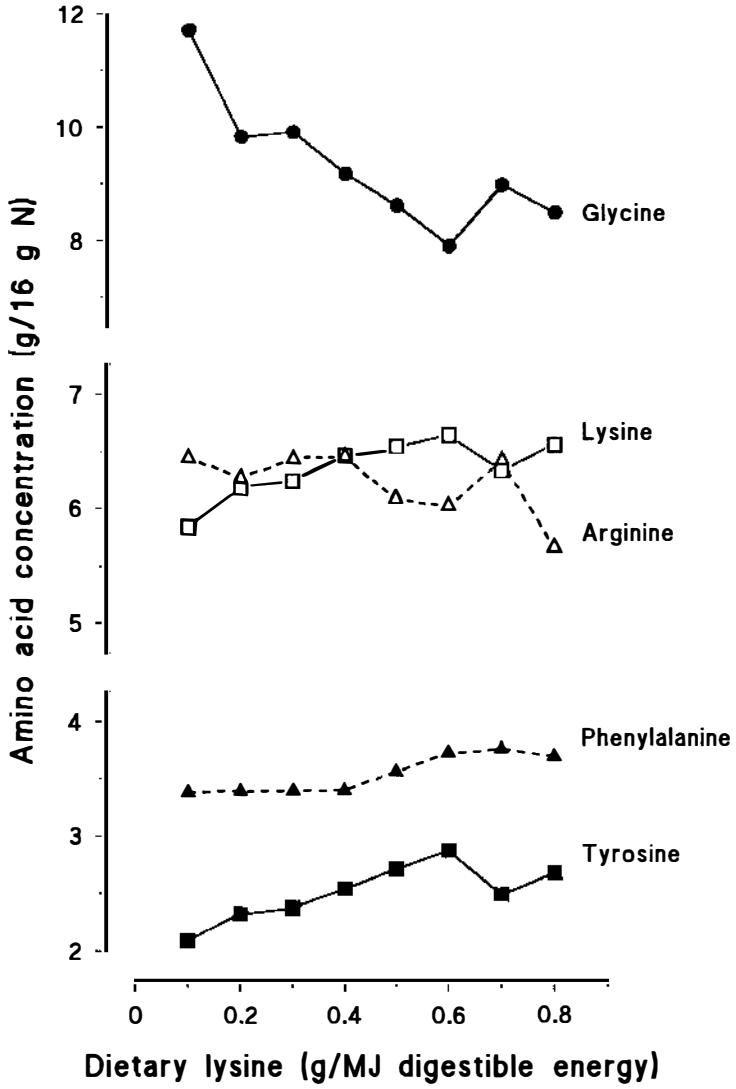

Figure 1 Changes in the amino acid composition of the whole-body protein of pigs given diets with various concentrations of lysine (2).

to maintain body protein on histidine-deficient diets. We suspect that similar mechanisms were operating in Rose's subjects.

Such adaptation to amino acid deficiency may not be unique to histidine. Evidence from experiments with animals indicates that substantial changes

may occur in the amino acid composition of the body when amino acid–deficient diets are given. For example, data given in Figure 1 show that pigs given diets low in lysine have less lysine per 16 g N in whole-body protein than those given an adequate diet. They also have less phenylalanine and tyrosine and more glycine and arginine (2). Gahl et al reported similar results (20). These data imply that, as with histidine, deficiency of an individual amino acid does not necessarily entail a proportionate loss of body protein and a commensurately negative nitrogen balance. These changes presumably result from alterations in the relative amounts of different body proteins, which in turn implies that the rates of synthesis or breakdown of these proteins are sensitive to dietary amino acid supply. However, the identity of the proteins involved and the mechanism for the control of their turnover are as yet undiscovered.

In their nitrogen balance studies Rose, Leverton, and their colleagues took the amino acid requirement to be that which was sufficient to maintain nitrogen equilibrium, which they considered to imply that there was no gain or loss of the amino acid in question. We do not know whether adult men and women adapt to dietary amino acid deficiencies by modifying the amino acid composition of their bodies in the same way as growing animals, but if they do one can expect estimates of amino acid requirements made by nitrogen balance to fall short of the amounts needed for true protein homeostasis. The long-term consequences for physiological function and health of such alterations in body amino acid composition have not been explored.

REQUIREMENTS FROM AMINO ACID OXIDATION

Amino acid oxidation can be viewed as an important evolutionary mechanism for disposing of intoxicating concentrations of amino acids resulting from the consumption of protein in excess of requirements (4). When the intake of any amino acid exceeds the rate of its disposal, plasma concentration rises and oxidation rate increases, augmented by activation or induction of the pertinent oxidative enzymes. Although in amino acid deficiency the limiting amino acid is highly conserved by suppressing the activities of oxidizing enzymes, some residual oxidation continues, which is the obligatory oxidative loss (OOL). Two distinct approaches have been used to estimate amino acid requirements through measurement of amino acid oxidation: the "break-point" and "tracer balance" methods. In addition, OOL has been estimated from obligatory nitrogen excretion.

Break-Point Analysis

The relationships between amino acid intake and oxidation have been used to assess requirements by using break-point analysis to estimate the dietary concentration, or daily intake, above which oxidation increases. An alternative

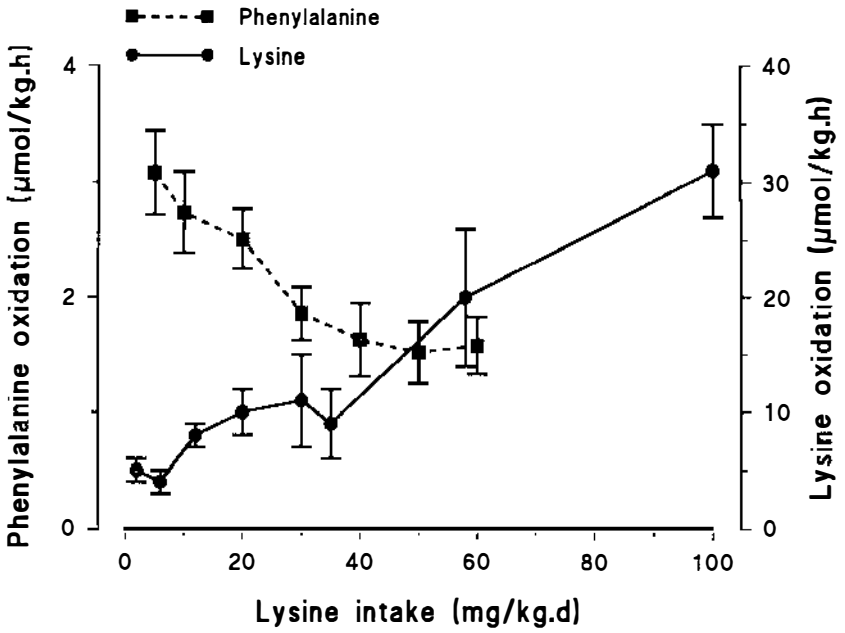

Figure 2 Effect of lysine intake on phenylalanine oxidation (± SE; 78) and lysine oxidation (± SD; 44) in adult males.

approach, which also uses amino acid oxidation, relies on the fact that, when the diet is deficient in one amino acid, the relative excesses of the other amino acids are necessarily oxidized. With increasing intake of the limiting amino acid, the oxidation of another amino acid diminishes progressively until the requirement for the limiting amino acid is met. This indirect indicator method, developed in animal experiments, has also been applied in human studies (78). Estimates of lysine requirements made using these two approaches are shown in Figure 2. The lysine requirement estimated by the indirect oxidation method was 37 mg kg^{-1} d^{-1}. The direct oxidation data do not show a distinct break point, but the increase in oxidation at lysine intakes over 35 mg kg^{-1} d^{-1} is not inconsistent with the indirect estimate, although a wide range of values is possible. In such studies, errors of measurement often make it difficult to establish a break point accurately.

Tracer Balance Method

The second method of deducing requirements from amino acid oxidation has been termed the "tracer balance" approach by Young and coworkers. In this approach it is assumed that oxidation is the only significant component of

amino acid loss, which reaches a minimum rate at low intakes and so determines the requirement. In this method the rate of oxidation of the test amino acid is measured directly using a (usually stable) isotope label. The requirement is defined as the dietary intake at which intake and oxidation are just balanced.

OXIDATION AS A COMPONENT OF REQUIREMENTS. Because the maintenance requirement is the sum of all obligatory amino acid losses, it is appropriate, before discussing the technical points involved in the measurement of oxidation, to consider amino acid oxidation in relation to the other main routes of amino acid loss. These are identified in Table 3: Estimates of minimum losses are available for only some of them.

Few measurements of amino acid oxidation have been made in subjects receiving diets devoid of the amino acid under investigation. However, measurements have been made with diets sufficiently low in a single amino acid to be considered effectively amino acid free. Some of these measurements are presented in Table 4.

There is little direct information on amino acid losses via the skin. The minimum dermal nitrogen loss of 120 mg d^{-1}, measured by Calloway et al

Table 3 Routes of obligatory amino acid loss

Irreducible oxidation
Losses from the skin
Losses via the GI tract
Urinary excretion
Irreversible modification
Synthesis of nonprotein substances

Table 4 Estimates of minimal oxidation rates of amino acids in normal adults

	Amino acid (mg kg^{-1} d^{-1})		
	Intake	Oxidation[a]	Reference
Lysine	2–6	15	44
Threonine	3–10	13	79
Valine	4	11	44
Leucine	4–8	22	44
	10	47[b]	6
	14	28[b]	13
Methionine	13	14	75
Phenylalanine	5–10	5	78

[a] Estimates are not adjusted for the labeled amino acid infused: see p. 229 for discussion.

[b] Using plasma KIC as a precursor.

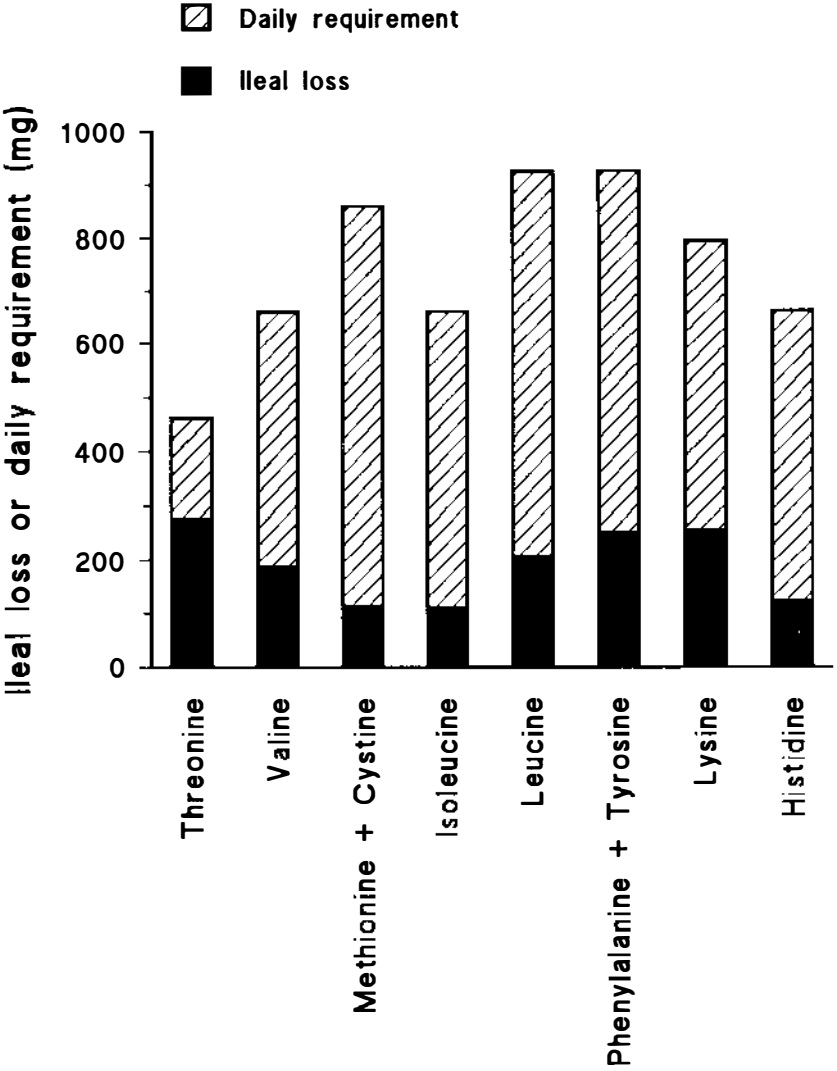

Figure 3 Mean daily losses of essential amino acids in ileostomy fluid (19), compared with current estimates of daily maintenance requirements for adults of the same weight (16).

(3), constitutes less than 5% of total adult maintenance nitrogen needs. Amino acid nitrogen does not account for all of this loss. The most important losses may be those of cystine in the keratins of skin, hair, and nails, which according to Calloway et al (3) may contribute 34–75 mg N d^{-1}. This amount would correspond to only ~ 5% of total sulphur amino acid needs for

maintenance. Amino acid losses from the gastrointestinal (GI) tract may be more substantial. We (19) measured amino acid losses in the ileostomy fluid of subjects fed a protein-free diet, which we consider similar to the flow of amino acids into the cecum of normal subjects. These losses amounted to some 15–30% of requirements [Food and Agricultural Organization (FAO)/World Health Organization (WHO)/United Nations University (UNU) estimates, 16], with the exception of threonine, for which the proportion was 60% (Figure 3; 19).

The urinary excretion of five amino acids was slightly sensitive to amino acid intake (37). Minimum rates were less than 5 mg d^{-1} for four of the five, but on a threonine-free diet over 20 mg of threonine were lost in urine. However, this amount constitutes less than 5% of the total requirement.

There are additional urinary losses of certain specific amino acids that are modified after incorporation into proteins, principally by methylation or hydroxylation, and are not available for reuse after release by protein breakdown. Perhaps the most important of these is 3-methyl histidine, a constituent of contractile proteins. The daily 3-methyl histidine excretion by adults on a meat-free diet is typically ~ 0.25 mmol (73), which accounts for ~ 5% of the daily histidine requirement estimated by FAO/WHO/UNU (16). Likewise, although physiologically essential, the use of amino acids as precursors for the synthesis of various substances, including hormones and neurotransmitters, probably accounts for only a trivial proportion of total requirements.

To calculate amino acid needs, all these losses must be summated. The largest component of obligatory loss is clearly oxidation, but GI losses are also important. However, the estimates of requirements made by tracer kinetics (13, 42–44, 79), which have been advanced in support of the suggestion that current estimates are too low, assume that the requirement is the intake needed to equal only the OOLs. The error introduced by this assumption depends on the fate of the carbon skeletons of amino acids that enter the colon. If the amino acid is oxidized by the bacteria or its carbon is absorbed and metabolized in the tissues, it may be included in measurements of labeled CO_2 production. However, if the amino acid is sequestered in microbial biomass, it constitutes an additional loss and results in an underestimate of the requirement. It would be important to have more information on this point.

MEASUREMENT OF AMINO ACID OXIDATION WITH CARBON-LABELED AMINO ACIDS The basic technique for measuring amino acid oxidation involves i.v. (or sometimes intragastric) infusion of a carbon-labeled amino acid, with determination of the excretion of the label as breath CO_2 and of the isotopic enrichment of the amino acid at the site of oxidation. Using [1-^{13}C]-

leucine, for example, the infusion results in the attainment of constant (plateau) values for the enrichment of plasma free leucine and respiratory $^{13}CO_2$. Assuming that these plateau values represent a simple precursor-product relationship, the rate of oxidation of the amino acid can then be calculated (26, 39, 64). The formula for calculating the rate of oxidation (O) is therefore

$$O = C/E. \qquad 1.^{1}$$

C is the rate of production of labeled CO_2, which is determined by measuring the production rate and isotopic enrichment of respiratory CO_2 and multiplying by a factor to allow for the retention of the label by CO_2 fixation in body tissues. E is the isotopic enrichment of the precursor, which was taken to be that of the plasma free amino acid in early studies of the requirements for leucine (42), valine (43), threonine (79), and lysine (44).

This approach has been criticized (48) because the plasma amino acid is not the direct precursor of oxidation. However, in the case of leucine, a more direct precursor of labeled CO_2 is accessible in the form of α-ketoisocaproate (KIC), the transamination product of leucine, which is thought to leak into the plasma from its site of formation, mainly in muscle (40). KIC enrichment is lower than that of plasma leucine, which results in higher estimates of oxidation rate. However, more recent studies employing KIC enrichment have not revealed any error in the earlier studies large enough to alter radically the estimates of leucine requirement (6, 13). Nevertheless, it should be noted that when calcu-

[1]An alternative formula for oxidation (39) has been used in some studies:

$$O = C.(1/E_p - 1/E_i), \qquad 2.$$

where E_p is the isotopic enrichment of the precursor and E_i is the isotopic enrichment of the amino acid infused. The difference between this formula and Equation 1 is the second term within the parentheses, which makes an adjustment to the oxidation rate for the infusion of substrate quantities of amino acid when stable isotopes are used (see next section). Equation 1 calculates the total rate of oxidation, including any increase that might have resulted from the infusion of substrate amounts of amino acid. The use of Equation 2 recognizes that the infusion cannot be given as a true tracer and that this additional amino acid must be disposed of, so it assumes that it is cleared via oxidation and other pathways (e.g. protein synthesis) in the proportions that the label enters these pathways. This implies that the rates of all these pathways are increased in order to remove the excess amino acid and, moreover, that they are all increased equally. We see no reason for this assumption, since oxidation and protein synthesis are regulated independently. Which pathways are stimulated to dispose of the additional substrate depends on whether the amino acid is limiting or in relative excess at the time. However, the solution to this issue has no bearing on the precision of the oxidation rates presented by Meguid et al (42, 43) and in other studies from the same group (6, 44, 53, 71, 79), even though they used Equation 2. The subsequent calculation added back the same amount that had been subtracted in Equation 2, which is equivalent to using Equation 1 to calculate total oxidation.

lations employ the plasma amino acid (e.g. for lysine and threonine which lack a convenient keto acid or equivalent), positive amino acid balances will be overestimated, leading to erroneously low estimates of requirement. As Millward & Rivers suggested (48), allowance for this source of error could as much as double the estimate of requirement.

INFUSION OF NUTRITIONALLY SIGNIFICANT AMOUNTS OF "TRACER" AMINO ACID

The method of measuring amino acid oxidation was originally developed with the radioisotope ^{14}C (26), which can be given as a genuine tracer. However, with stable isotopes the methods of detection are less sensitive, and it is necessary to infuse an amount of labeled amino acid that is significant in relation to oxidation or dietary intake. For example, Millward (46) pointed out that in studies of lysine requirement (44), the rate of [^{13}C]lysine infusion was equal to three times the rate of lysine intake from the diet at the lowest dietary intake studied. This problem is unavoidable with stable isotopes, but it has a number of implications which must be evaluated.

When calculating the amino acid balance, investigators have treated the infusion of tracer similarly to dietary intake. Thus, amino acid balance is calculated as the intake from the diet (which is zero during fasting) plus the intake from the infusion minus the rate of oxidation. However, several questions arise about the exact way in which this is done. The first concerns how to extrapolate from a measurement period of a few hours, involving both fed and fasted states, to the balance over an entire 24-h day. The procedure that has been used is to divide the day into two notional periods of 12 h, one fed and one fasted. The hourly oxidation rate during each short measurement period is then multiplied by 12, and the values are added together to obtain the total daily oxidation. In calculating the balance, however, only the labeled amino acid intake given during the period of infusion was included (e.g. 6, 42–44), which we believe to be inappropriate, the correct method being to calculate the balance during the measurement period and to scale that value up to 12 h. Recalculation by this method shows balances that are more positive by a variable but significant amount depending on the experimental protocol. For example, the underestimates of leucine balance are 9 mg kg^{-1} d^{-1} (71) and 13 mg kg^{-1} d^{-1} (6) and that for lysine ~ 8 mg kg^{-1} d^{-1} (44).

A second problem resulting from the infused amino acid is that on the day of measurement an additional amount of the test amino acid was administered. Thus the day of infusion differed from the previous days during which the volunteers adapted to the particular intake under study. Do we assume that a balance measured at a particular nominal intake of the test amino acid (i.e. the adapted intake) is appropriate to that nominal intake or to the higher intake on

the day of measurement? The answer to this question depends on how quickly the body adapts to the new intake. This might have resulted in a small underestimate of requirement. In 24-h studies of leucine oxidation, El-Khoury et al (13) attempted to solve this problem by omitting from the diet on the day of the infusion an amount of leucine equal to that infused, so that the intake on the infusion day was the same as the intake during adaptation. However, this procedure results in additional problems of interpretation. Because the infusion was given for 24 h, but the diet for only 10 h, only 10/24 of the daily leucine infused was given during the absorptive period, so the diet supplied less leucine than the nominal intake. For example, at the lowest dietary level of 14 mg kg^{-1} d^{-1}, the actual intake during the feeding period was reduced to ~ 60% of the nominal level for the diet. Whether the remainder of the leucine infused during the fasting period could have counteracted this deficiency is questionable, because a limiting amino acid given separately from the remainder of the protein would not be well utilized. Much of the additional leucine infused during fasting would probably have been oxidized. This view is supported by the data obtained by El-Khoury et al (13), which show a gradual increase in leucine oxidation during the fasting period, as the excess was oxidized, and a fall during feeding due to the high efficiency of utilization on the very low-leucine diet.

Although the diet could be modified so that the total leucine intake during the feeding period would equal the nominal intake, it is still necessary to make appropriate allowance for the substrate quantities of leucine given during the fasting period. Whether this additional amino acid can reduce the net loss of protein that occurs during fasting or whether it is quantitatively oxidized remains unknown. Moreover, the excess might activate the enzymes responsible for oxidation and thus influence its utilization in the fed state.

As this discussion indicates, the need to give a nutritionally significant amount of amino acid for stable isotope labeling introduces a series of difficulties, which might be completely avoided only by resorting to a radioactive label (^{14}C or ^{3}H). From the data currently available, however, it seems that this problem is likely to have given rise to an appreciable overestimate of the amino acid requirement.

FOOD INTAKE IN RELATION TO THE MEASUREMENT OF OXIDATION In order to measure amino acid balance over an entire day, one must include a normal dietary intake. However, this conflicts with the need to maintain a metabolic steady state during the isotope infusion in order to achieve a steady plateau, which has been assumed to preclude the consumption of large meals. This dilemma has generally been resolved by giving small meals at regular (hourly) intervals during a period that can be designated as fed (21, 26). Recognizing that normal adults spend part of the day fed and part of the day in the

postabsorptive state, recent studies of requirements have included measurements during short periods (3–5 h) of both fasting and feeding (e.g. 6, 71). The difficulty inherent in such studies relates to that discussed earlier and arises from the need to extrapolate results from these two short periods to obtain the amino acid balance over an entire day. Conceptually the day is divided into 2 periods of 12 h each, represented by the 2 measurement periods (e.g. 6, 44), with each of the small meals comprising 1/12 of the total daily intake. Millward & Rivers criticized this procedure (48) based on the lack of evidence that these two periods are fully representative; typically the fasting period was in the morning after an overnight fast (i.e. 12–15 h postabsorptive), whereas the chosen feeding period was after 3–5 h of small meals. A recent study demonstrated that these 2 periods were indeed well chosen when the diet contained a surplus of leucine, resulting in a very similar estimate of leucine balance to that from a complete 24-h measurement (12). However, when the diet contained just adequate or inadequate amounts of leucine according to the requirements proposed by Young et al (68, 69, 74), the short periods yielded an overestimate by ~ 3–5 mg kg^{-1} d^{-1} of the total daily oxidation (13), suggesting that the requirements determined in earlier studies using the shorter protocol might have been somewhat high.

The assumption that the day can be divided into two 12-h periods can, however, give rise to error when the meals do not each contain 1/12 of the total daily intake (e.g. 12, 13, 71). For example, in a study of leucine metabolism in the fed and fasted states (71), the diet during the fed infusion was given at a rate of 1/8 of the daily intake each hour. The leucine oxidation values obtained during this fed period should then have been multiplied by eight to account for a complete day's intake. The conceptual day in this case should therefore be calculated on the basis of 8 h of feeding and 16 h of fasting. The authors used 12 h for each, which resulted in an overestimate of the total daily oxidation by ~ 5 mg kg^{-1} d^{-1} in subjects given 30 mg kg^{-1} d^{-1} leucine for 1 week. The difference between the two methods of calculation is much smaller with the very low leucine diets because there is then little difference between the fed and fasted rates of oxidation. In this study, therefore, the method of calculation probably resulted in a slight overestimate of the leucine requirement.

Overall, the influence of the feeding pattern during the isotope studies on the estimates of requirement appears to be quite small, in the range 0–5 mg kg^{-1} d^{-1} too high for leucine. However, the apparent need to give small meals clearly imposes practical and computational problems. Moreover, the impact of the change in dietary pattern from a regime of relatively large meals during the adaptation period to one of frequent small meals during the measurement is unknown. This factor, coupled with the fact that the subjects are restrained from normal activity during the period of infusion, may well result in an

atypically negative daily balance. If we accept the need for a steady state, these difficulties are largely unavoidable, but perhaps we should question whether such a well-controlled steady state is really necessary. The method for determining oxidation involves measurement of both precursor and product. Theoretically this does not rely on a steady state if sufficient measurements are made and if each rate of $^{13}CO_2$ production can be related to a precursor measurement at the same time. The main problem is that there is a delay in the appearance of $^{13}CO_2$ in the breath as a result of the ~ 45-min half-life of the body bicarbonate pool. However, this would only cause serious errors if there were a pronounced and rapidly changing metabolic state, which would probably not occur with normal meals. To avoid errors resulting from the pattern of food intake during the isotope infusion, it is probably more important to avoid unnecessary activity and to make measurements over a complete 24-h period.

From the above discussion it is evident that there are several real and potential errors in the tracer balance method of estimating requirements. The most readily quantified and consistent factor is the underestimate of intake from the stable isotope infusion. Recalculation of published data leads to much lower estimates of requirements, e.g. lysine is in balance at 12–15 mg kg^{-1} d^{-1} rather than at 20–30 mg kg-1 d-1 (44) and leucine at 20–30 mg kg^{-1} d^{-1} instead of ~ 40 mg kg^{-1} d^{-1} (6). In these examples, the other potential errors have probably resulted in underestimates of requirement. The most critical of these errors are: (*a*) other routes of loss were not considered and (*b*) in the case of lysine, the precursor enrichment derived from plasma lysine was too high. We cannot judge how these opposing sources of error may have balanced out, but it seems likely that requirements estimated in this way are indeed substantially higher than the FAO/WHO/UNU (16) recommendations.

AMINO ACID REQUIREMENTS DEDUCED FROM OBLIGATORY NITROGEN LOSS

On a protein-free diet, but with an ample energy supply, the amino acids utilized for the various processes that constitute basal protein metabolism are provided by the dissimilation of body protein, giving rise to a basal or obligatory rate of nitrogen loss (ONL) which is remarkably constant across species, approximating 2 mg per basal calorie (see review by Munro, 50). In humans, this loss is taken to be 54 mg kg^{-1} d^{-1} (16). Millward & Rivers (48) suggested that "the oxidation rates of amino acids which give rise to the ONL, the obligatory oxidative losses (OOL), should equal the amino acid content of the tissue protein being mobilised." Young, Bier & Pellett (70) took this to mean that requirements could be estimated by assuming that individual amino acids are oxidized in proportion to their concentrations in mixed body proteins. These oxidative losses of amino acids are indeed incurred on a protein-free diet but,

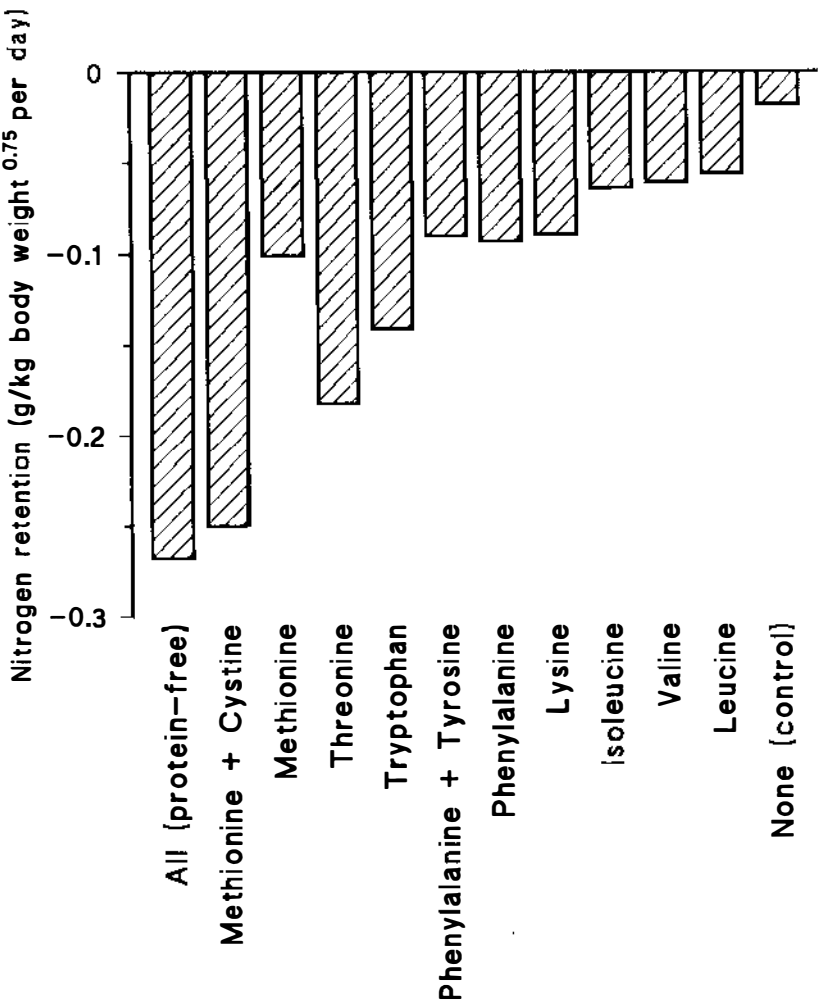

Figure 4 Body nitrogen losses in young pigs when individual amino acids were deleted from otherwise complete diets (18).

as Millward et al (47) point out, this does not mean that they are the *minimal* rates of oxidation. On a protein-free diet the net catabolism of body protein is that needed to meet the obligatory disposal of whichever amino acid is in relatively greatest demand.

Our own results with pigs (18) show that on a protein-free diet the need for sulphur amino acids apparently determines the rate of body protein loss. In these experiments pigs were given diets devoid of a single amino acid but adequate in all others. The rate of body nitrogen loss when methionine and

cystine were omitted was almost as great as when a protein-free diet was given (Figure 4). Deletion of threonine also provoked a high rate of nitrogen loss. These findings are consistent with the observation (66) that simultaneous addition of methionine and threonine to a protein-free diet spares a disproportionate amount of body protein. In contrast, the deletion of lysine and leucine, which are relatively abundant in tissue protein, resulted in little body nitrogen loss (Figure 4), indicating relatively low rates of obligatory disposal. Although, as Young (67, 68) has argued, it is unreasonable to apply quantitative information from growing pigs to adult men, it seems certain that this same principle applies.

There is thus no particular connection between the proportions of amino acids in the body and their relative rates of obligatory disposal. As well as the predominance of the sulphur amino acids and threonine, another striking feature of maintenance amino acid needs is the very low ratio of indispensable to dispensable amino acids. In the experiments with pigs described above, for example, this ratio was less than 20:80. This observation could of course result from an underestimate of indispensable amino acid requirements. However, supporting evidence comes from the experiments of Kofrányi & Jekat (29), who showed that egg protein could be diluted isonitrogenously 40:60 with dispensable amino acids or diammonium citrate without increasing the total nitrogen requirement. In such a mixture the indispensable amino acids provided only ~ 20% of the total nitrogen.

OTHER FACTORS INFLUENCING REQUIREMENTS

Any discussion of amino acid requirements must consider what is meant by "requirement." This issue includes two separate questions: (*a*) What is the criterion of adequacy and for what population? (*b*) Is the diet the sole source of amino acids to meet metabolic requirements?

Criteria of Adequacy

As described above, estimates of amino acid requirements were until recently based on experiments in which nitrogen balance was the criterion of adequacy. Such studies involve adaptations to conserve the limiting amino acid. It is important to note (although it is not an issue in the present controversy) that little is known about the impact of such adaptations on other physiological functions or long-term health. However, there is now evidence to suggest that certain amino acids may have special roles in the competence of the immune system (1, 51), and it would be important to understand how intakes of amino acids greater than those required for protein homeostasis may modify more subtly the physiological responses of the body in regard to health and fitness.

Adaptation is one of the important issues in the design and interpretation of nutritional experiments because of the time required for subjects to become fully adapted. In many experiments, subjects were fed diets with various levels of the limiting amino acid in a rather rapid sequence. In the experiments on lysine requirements by Zello et al (78), for example, the subjects were given a generous level of lysine (60 mg kg^{-1} d^{-1}) except on the days of measurement, when various intakes (5–60 mg kg^{-1} d^{-1}) were given. As Young (68) has suggested, the previous level of amino acid intake may affect the response observed during any period of measurement; recent studies by this group have introduced periods with an adequate intake between periods with depleted diets (e.g. 6). Moreover, the duration of adaptation is important. For example, Young et al (71) showed that subjects given 7 or 14 mg of leucine kg^{-1} d^{-1} were in negative leucine balance after 1 week but were almost in balance after 3 weeks. Young & Marchini (72) have suggested that balance at three weeks was achieved through an accommodation rather than an adaptation, which might be detrimental to health because rates of whole-body protein turnover were depressed. However, the consequences for health and longevity of such alterations of metabolism are unknown.

One must also distinguish between the minimum physiological requirement of an individual and the "safe practical allowance" recommended for the population at large. This distinction focuses on the statistical distribution of requirements within the population. The FAO/WHO/UNU Expert Committee (16) assumed that the coefficient of variation of adult protein needs is 12.5%, so that an excess of 2 standard deviations would satisfy the needs of 95% of the population. This was defined as a safe protein intake for healthy adults. Therefore, at the safe protein intake level some individuals will receive 50% more protein than they need. However, without further characterization of individuals in defined subsets with different protein needs, such global definitions of requirements seem unavoidable.

Amino Acid Production in the GI Tract

It is normally assumed that the diet is the only source of indispensable amino acids to satisfy metabolic needs, and all current estimates of requirements are based on this assumption. Ruminant animals obtain a large proportion of their amino acid requirements from the digestion of microbial protein synthesized in the rumen, whereas in nonruminants sufficient microbial activity for the biosynthesis of nutritionally significant quantities of amino acids is generally thought to occur only in the large intestine, beyond the sites of significant amino acid absorption. Support for this view comes from experiments in which protein (28, 76, 77) or amino acids (65) infused into the terminal ileum or cecum promoted little or no response, in contrast to the same supplements

given orally. On the other hand, evidence from studies with animals (8, 9) and human subjects (22, 61) given ^{15}N-labeled ammonia or urea indicates that microbial amino acids are absorbed.

Of course, the mere appearance of labeled protein in the body after cecal administration of labeled proteins (25) does not of itself imply the absorption of microbial amino acids. Most amino acids in the body can acquire ^{15}N by transamination and, with a label such as ^{15}N ammonium compounds, the degree of labeling of amino acids relates to transaminase activity. Two amino acids however, lysine and threonine, do not engage in transamination reactions (14, 17), and any labeling in these can be interpreted as evidence of microbial amino acid absorption. In the studies with pigs referred to above (9), lysine and threonine were among the amino acids in the body that acquired the ^{15}N label. Curiously, this labeling also occurred in a germ-free animal, although few details were given about the methods or the tests made to confirm the animal's germ-free status. Studies of human subjects (61) also showed labeling of lysine to no less an extent than other amino acids. Labeling of nitrogen in the imidazole ring of histidine (59) can also be attributed to microbial biosynthesis. Alternatively, carbon-labeled substrates can be given; in this case, any carbon-labeled indispensable amino acid in the tissues (with the exception of methyl-labeled methionine) can only be derived from the GI microflora. However, none of these studies allowed the quantitative significance of this route of indispensable amino acid supply to be quantified.

Recent studies (62, 63) in rats and pigs with both ^{15}N- and ^{14}C-labeled substrates have involved simultaneous measurement of the labeling of amino acids in the body and in microbial protein from the digesta, thereby allowing the transfer to be estimated. The data suggest that, in these species, the GI microflora may provide nutritionally significant quantities of amino acids. In the rat, labeling was abolished when coprophagy was prevented (D Torrallardona, unpublished observations), but this was not so in the pig. There was no labeling of lysine in germ-free rats (63), confirming that this amino acid does not undergo transamination. These results provide new evidence of the importance of the GI microflora in making available additional quantities of indispensable amino acids. The significance of this supply in man and how its magnitude may vary with nutritional conditions and adaptation are not yet known, but if it is subsequently shown to be significant, this will provide further resolution of the controversy, because amino acid disposal might then be equated with the sum of dietary and microbial supplies. Of course, this phenomenon differs from that discussed by Oomen (52), who was concerned with the ability of people to remain healthy while living on very low nitrogen intakes. That adaptation involves the conservation of total nitrogen rather than of individual amino acids.

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

Having examined the protocols and assumptions in both the nitrogen balance and the tracer experiments, we conclude that neither approach is clearly better than the other. That both have defects does not diminish the invaluable contributions that the respective authors have made to our understanding of these complex issues. What does appear clear to us is that the original nitrogen balance experiments, with both men and women subjects, almost certainly overestimated nitrogen retention and thereby underestimated amino acid requirements. Even the values from tracer experiments may have underestimated the total losses in two ways: (*a*) by considering oxidation the only significant route of amino acid loss and (*b*) for amino acids other than leucine, by using the plasma amino acid to determine the precursor enrichment. The tracer balance method has sources of error, the largest of which would have resulted in an overestimate of losses and hence requirements. Yet even when these errors are taken into account, the apparent requirements remain higher than those derived from N balance. Thus we can find no evidence to suggest that requirements derived by the tracer balance technique have been grossly overestimated. However, before a definite answer can be given, several important issues must be addressed in future work:

1. We need to know more about amino acid metabolism in the GI tract, both about the unrecovered losses of endogenous amino acids and about the role of microbial biosynthesis in providing amino acids to meet the needs of the host.
2. The methodology of measuring amino acid oxidation with stable isotopes needs to be more fully validated. This is especially important at low intakes, when the amount of amino acid infused is of the same order as the requirement. The use of ^{14}C-labeled amino acids should be considered. Moreover, with the exception of those experiments with leucine in which KIC enrichment was measured, oxidation has been underestimated through failure to measure the enrichment of the direct precursor for oxidation. Such direct measurements are needed for all amino acids.
3. More information is needed about the changing temporal pattern of amino acid oxidation during a normal day with its sequence of meals and overnight fast.
4. We need to know to what extent the amino acid composition of the body may change in response to alterations in amino acid intake. This may vary from one amino acid to another.
5. Finally, the criteria of amino acid adequacy should not be confined to short-term metabolic or nutritional responses; different habitual intakes of amino acids may carry important health implications.

Some argue that normal western diets are so rarely deficient in any amino acid that the answers to these questions are at best of value only to planners of nutrition programs for developing nations and at worst merely of academic interest. We respond to this view with the following question: Is it acceptable that we humans, with our vast collection of scientific knowledge, should enter the twenty-first century still ignorant of something as basic as what our bodies require for sustenance?

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Literature Cited

1. Barbul A. 1990. Arginine and immune function. *Nutrition* 6:S56–S62
2. Batterham ES, Andersen LM, Baigent DR, White E. 1990. Utilization of ileal digestible amino acids by growing pigs: effect of dietary lysine concentration on efficiency of lysine retention. *Br. J. Nutr.* 64:81–94
3. Calloway DH, Odell ACF, Margen SJ. 1971. Sweat and miscellaneous nitrogen losses in human balance studies. *J. Nutr.* 101:775–86
4. Carpenter KJ. 1992. Protein requirements of adults from an evolutionary perspective. *Am. J. Clin. Nutr.* 55:913–17
5. Chung-Hwang E, Khurana H, Fisher H. 1976. The effect of dietary histidine level on the carnosine concentration of rat olfactory bulbs. *J. Neurochem.* 26: 1087–91
6. Cortiella J, Matthews DE, Hoerr R, Bier DM, Young VR. 1988. Leucine kinetics at graded intakes in young men: quantitative fate of dietary leucine. *Am. J. Clin. Nutr.* 48:998–1009
7. Costa G, Ullrich L, Kantor F, Holland JF. 1968. Production of elemental nitrogen by certain mammals. *Nature* 218: 546–51
8. Deguchi E, Niiyama M, Kagota K, Namioka S. 1978. Incorporation of ^{15}N administered to germfree and SPF piglets as ^{15}N-urea into amino acids of hydrolyzed liver and muscle proteins. *Jpn. J. Vet. Res.* 26:68–73
9. Deguchi E, Niiyama M, Kagota K, Namioka S. 1980. Incorporation of nitrogen-15 from dietary [^{15}N]-diammonium citrate into amino acids of liver and muscle proteins in germfree and specific-pathogen-free neonatal pigs. *Am. J. Vet. Res.* 41:212–14
10. Duncan DL. 1958. The interpretation of studies of calcium and phosphorus balance in ruminants. *Nutr. Abs. Rev.* 28: 695–715
11. Easter RA, Baker DH. 1977. Nitrogen metabolism, tissue carnosine concentration and blood chemistry of gravid swine fed graded levels of histidine. *J. Nutr.* 107:120–25
12. El-Khoury AE, Fukagawa NK, Sánchez M, Tsay RH, Gleason RE, et al. 1994. Validation of the tracer balance concept with reference to leucine: 24 hour intravenous tracer studies with L-(1-^{13}C) leucine and (^{15}N-15N) urea. *Am. J. Clin. Nutr.* In press
13. El-Khoury AE, Fukagawa NK, Sánchez M, Tsay RH, Gleason RE, et al. 1994. The 24 hour pattern and rate of leucine oxidation, with particular reference to tracer estimates of leucine requirements in healthy adults. *Am. J. Clin. Nutr.* In press
14. Elliott DF, Neuberger A. 1950. The irreversibility of the deamination of thre-

onine in the rabbit and rat. *Biochemistry* 46:207–10
15. Equality of inspired and expired N_2 in man. 1974. *Nutr. Rev.* 32:117–20
16. FAO/WHO/UNU. 1985. *Energy and Protein Requirements. Tech. Rep. Ser. 724*. Geneva: WHO
17. Foster GL, Schoenheimer R, Rittenberg D. 1939. Studies in protein metabolism. V. The utilization of ammonia for amino acid and creatine formation in animals. *J. Biol. Chem.* 127:319–27
18. Fuller MF, McWilliam R, Wang TC, Giles LR. 1989. The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. *Br. J. Nutr.* 62:255–67
19. Fuller MF, Milne A, Harris CI, Reid TM, Keenan R. 1994. Amino acid losses in ileostomy fluid on a protein-free diet. *Am. J. Clin. Nutr.* 59:70–73
20. Gahl MJ, Crenshaw TD, Benevenga NJ. 1992. Amino acid composition in growing pigs fed graded levels of lysine. In *Proc. Am. Soc. Anim. Sci.*, p. 66. Des Moines (Abstr.)
21. Garlick PJ, Clugston GA, Swick RW, Waterlow JC. 1980. Diurnal pattern of protein and energy metabolism in man. *Am. J. Clin. Nutr.* 33:1983–86
22. Giordano C, DePascale C, Balestrieri C, Cittadini D, Crescenzi A. 1968. The incorporation of urea ^{15}N in amino acids of patients with chronic renal failure on low nitrogen diet. *Am. J. Clin. Nutr.* 21:394–404
23. Hegsted DM. 1963. Variation in requirements of nutrients-amino acids. *Fed. Proc.* 22:1424–30
24. Hegsted DM. 1976. Balance studies. *J. Nutr.* 106:307–11
25. Heine W, Wutzke KD, Richter I, Walther F, Plath C. 1987. Evidence for colonic absorption of protein nitrogen in infants. *Acta Paediatr. Scand.* 76: 741–44
26. James WPT, Sender PM, Garlick PJ, Waterlow JC. 1974. The choice of label and measurement technique in tracer studies in man. In *Dynamic Studies with Radioisotopes in Medicine*, 1:461–72. Vienna: IAEA
27. Jones EM, Baumann CA, Reynolds MS. 1956. Nitrogen balances of women maintained on various levels of lysine. *J. Nutr.* 60:549–62
28. Just A, Jørgensen H, Fernández JA. 1981. The digestive capacity of the caecum-colon and the value of the nitrogen absorbed from the hind gut for protein synthesis in pigs. *Br. J. Nutr.* 46:209–19
29. Kofrányi E, Jekat F. 1964. Determination of the biological value of food proteins. IX. Substitution of high-value proteins by non-essential nitrogen. *Z. Physiol. Chem.* 338:154–58
30. Kopple JD, Swendseid ME. 1975. Evidence that histidine is an essential amino acid in normal and chronically uremic man. *J. Clin. Invest.* 55:881–91
31. Leverton RM. 1959. Amino acid requirements of young adults. In *Protein and Amino Acid Nutrition*, ed. AA Albanese, pp. 477–506. New York: Academic Press
32. Leverton RM, Ellison J, Johnson N, Pazur J, Schmidt F, Geschwender D. 1956. The quantitative amino acid requirements of young women. V. Leucine. *J. Nutr.* 58:355–65
33. Leverton RM, Gram MR, Brodovsky E, Chaloupka M, Mitchell A, Johnson N. 1956. The quantitative amino acid requirements of young women. II. Valine. *J. Nutr.* 58:83–93
34. Leverton RM, Gram MR, Chaloupka M, Brodovsky E, Mitchell A. 1956. The quantitative amino acid requirements of young women. I. Threonine. *J. Nutr.* 58:59–81
35. Leverton RM, Johnson N, Ellison J, Geschwender D, Schmidt F. 1956. The quantitative amino acid requirements of young women. IV. Phenylalanine, with and without tyrosine. *J. Nutr.* 58:341–53
36. Leverton RM, Johnson N, Pazur J, Ellison J. 1956. The quantitative amino acid requirements of young women. III. Tryptophan. *J. Nutr.* 58:219–29
37. Leverton RM, Waddill FS, Skellenger M. 1959. The urinary excretion of five essential amino acids by young women. *J. Nutr.* 67:19–28
38. Mammalian production of N_2. 1968. *Nutr. Rev.* 26:308–11
39. Matthews DE, Motil KJ, Rohrbaugh D, Burke JF, Young VR, Bier DM. 1980. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C]-leucine. *Am. J. Physiol.* 238:E473–79
40. Matthews DE, Schwartz HP, Young RD, Motil KJ, Young VR. 1982. Relationship of plasma leucine and α-ketoisocaproate during a L-[1-^{13}C]leucine infusion in man: a method for measuring human intracellular leucine tracer enrichment. *Metabolism* 31:1105–12
41. McCoy RH, Meyer CE, Rose WC. 1935. Feeding experiments with mixtures of highly purified amino acids. VIII. Isolation and identification of a new essential amino acid. *J. Biol. Chem.* 112: 283–302
42. Meguid MM, Matthews DE, Bier DM,

Meredith CN, Soeldner JS, Young VR. 1986. Leucine kinetics at graded leucine intakes in young men. *Am. J. Clin. Nutr.* 43:770–80
43. Meguid MM, Matthews DE, Bier DM, Meredith CN, Young VR. 1986. Valine kinetics at graded valine intakes in young men. *Am. J. Clin. Nutr.* 43:781–86
44. Meredith CN, Wen Z-M, Bier DM, Matthews DE, Young VR. 1986. Lysine kinetics at graded lysine intakes in young men. *Am. J. Clin. Nutr.* 43:787–94
45. Meyer CE, Rose WC. 1936. The spatial configuration of α-amino-β-hydroxy-n-butyric acid. *J. Biol. Chem.* 115:721–29
46. Millward DJ. 1994. Can we define indispensable amino acid requirements and assess protein quality in adults? *J. Nutr.* In press
47. Millward DJ, Price GM, Pacy PJH, Halliday D. 1990. Maintenance protein requirements: the need for conceptual re-evaluation. *Proc. Nutr. Soc.* 49:473–87
48. Millward DJ, Rivers JPW. 1988. The nutritional role of indispensable amino acids and the metabolic basis for their requirements. *Eur. J. Clin. Nutr.* 42: 367–93
49. Munro HN. 1964. Historical introduction: the origin and growth of our present concepts of protein metabolism. In *Mammalian Protein Metabolism*, ed. HN Munro, JB Allison, pp. 1–29. London: Academic Press
50. Munro HN. 1964. General aspects of the regulation of protein metabolism by diet and hormones. See Ref. 49, pp. 381–481.
51. Newsholme EA, Crabtree B, Ardawi MSM. 1985. Glutamine metabolism in lymphocytes: its biochemical, physiological and clinical importance. *Q. J. Exp. Physiol.* 70:473–89
52. Oomen HAPC. 1970. Interrelationship of the human intestinal flora and protein utilization. *Proc. Nutr. Soc.* 29:197–206
53. Pelletier V, Marks L, Wagner DA, Hoerr RA, Young VR. 1991. Branched-chain amino acid interactions with reference to amino acid requirements in adult men: valine metabolism at different leucine intakes. *Am. J. Clin. Nutr.* 54:395–401
54. Reynolds MS, Steel DL, Jones EM, Baumann CA. 1958. Nitrogen balances of women maintained on various levels of methionine and cystine. *J. Nutr.* 64: 99–111
55. Rose WC. 1937. The nutritive significance of the amino acids and certain related compounds. *Science* 86:298–300
56. Rose WC. 1957. The amino acid requirements of adult man. *Nutr. Abs. Rev.* 27:631–47
57. Rose WC, Cox GJ. 1924. The relation of arginine and histidine to growth. *J. Biol. Chem.* 61:747–73
58. Rose WC, Oesterling MJ, Womack M. 1948. Comparative growth on diets containing ten and nineteen amino acids, with further observations upon the role of glutamic and aspartic acids. *J. Biol. Chem.* 176:753–62
59. Sheng Y-B, Badger TM, Asplund JM, Wixom RL. 1977. Incorporation of $^{15}NH_4Cl$ into histidine in adult man. *J. Nutr.* 107:621–30
60. Swendseid ME, Dunn MS. 1956. Amino acid requirements of young women based on nitrogen balance data. II. Studies on isoleucine and on minimum amounts of the eight essential amino acids fed simultaneously. *J. Nutr.* 58: 507–17
61. Tanaka N, Kubo K, Shiraki K, Koishi H, Yoshimura H. 1980. A pilot study on protein metabolism in the Papua New Guinea highlanders. *J. Nutr. Sci. Vitaminol.* 26:247–59
62. Torrallardona D, Harris CI, Milne E, Fuller MF. 1993. Contribution of intestinal microflora to lysine requirements in nonruminants. *Proc. Nutr. Soc.* 52: 153A (Abstr.)
63. Torrallardona D, Harris CI, Milne E, Ronaasen V, Coates ME, Fuller MF. 1993. Contribution of intestinal microflora to lysine requirements in rats. *Proc. Nutr. Soc.* 52:368A
64. Waterlow JC, Garlick PJ, Millward DJ. 1978. *Protein Turnover in Mammalian Tissues and in the Whole Body.* Amsterdam: North Holland
65. Wünsche J, Hennig U, Meinl M, Bock HD. 1984. Amino acid absorption from the caecum of growing pigs. In *Proc. 6th Int. Symposium on Amino Acids*, ed. T Zebrowska, L Buraczewska, S Buraczewska, J Kowalczyk, B Pastuzewska, pp. 158–67. Warsaw: Pol. Sci. Publ.
66. Yoshida A, Moritoki K. 1974. Nitrogen sparing action of methionine and threonine in rats receiving a protein free diet. *Nutr. Rep. Int.* 9:159–68
67. Young VR. 1991. Nutrient interactions with reference to amino acid and protein metabolism in non-ruminants; particular emphasis on protein-energy relations in man. *Z. Ernähr.wiss.* 30:239–67
68. Young VR. 1992. Protein and amino acid requirements in humans: metabolic basis and current recommendations. *Scand. J. Nutr.* 36:47–56
69. Young VR, Bier DM. 1987. *Amino acid*

requirements in the adult human: How well do we know them? J. Nutr. 117: 1484–87
70. Young VR, Bier DM, Pellet PL. 1989. A theoretical basis for increasing current estimates of the amino acid requirements in adult man, with experimental support. *Am. J. Clin. Nutr.* 50:80–92
71. Young VR, Gucalp C, Rand WM, Matthews DE, Bier DM. 1987. Leucine kinetics during three weeks at submaintenance-to-maintenance intakes of leucine in men: adaptation and accommodation. *Hum. Nutr. Clin. Nutr.* 41C: 1–18
72. Young VR, Marchini JS. 1990. Mechanisms and nutritional significance of metabolic responses to altered intakes of protein and amino acids with reference to nutritional adaptation in humans. *Am. J. Clin. Nutr.* 51:270–89
73. Young VR, Munro HN. 1978. N^{τ}-methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. *Fed. Proc.* 37:77–86
74. Young VR, Pellet PL. 1990. Current concepts concerning indispensable amino acid needs in adults and their implications for international nutrition planning. *Food Nutr. Bull.* 12:289–300
75. Young VR, Wagner DA, Burini R, Storch KJ. 1991. Methionine kinetics and balance at the 1985 FAO/WHO/UNU intake requirement in adult men studied with L-[$^{2}H_{3}$-methyl-1-^{13}C]methionine as a tracer. *Am. J. Clin. Nutr.* 54:377–85
76. Zebrowska T. 1973. Digestion and absorption of nitrogenous compounds in the large intestine of pigs. *Rocz. Nauk Roln.* 95B:85–90
77. Zebrowska T. 1975. The apparent digestibility of nitrogen and individual amino acids in the large intestine of pigs. *Rocz. Nauk Roln.* 97B:117–23
78. Zello GA, Pencharz PB, Ball RO. 1993. Dietary lysine requirement of young adult males determined by oxidation of L-[1-^{13}C]phenylalanine. *Am. J. Physiol.* 264:E677–85
79. Zhao X-H, Wen Z-M, Meredith CN, Matthews DE, Bier DM, Young VR. 1986. Threonine kinetics at graded threonine intakes in young men. *Am. J. Clin. Nutr.* 43:795–802