Human Protein and Amino Acid Requirements

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Abstract

Human protein and amino acid nutrition encompasses a wide, complex, frequently misunderstood, and often contentious area of clinical research and practice. This tutorial explains the basic biochemical and physiologic principles that underlie our current understanding of protein and amino acid nutrition. The following topics are discussed: (1) the identity, measurement, and essentiality of nutritional proteins; (2) the definition and determination of minimum requirements; (3) nutrition adaptation; (4) obligatory nitrogen excretion and the minimum protein requirement; (5) minimum versus optimum protein intakes; (6) metabolic responses to surfeit and deficient protein intakes; (7) body composition and protein requirements; (8) labile protein; (9) N balance; (10) the principles of protein and amino acid turnover, including an analysis of the controversial indicator amino acid oxidation technique; (11) general guidelines for evaluating protein turnover articles; (12) amino acid turnover versus clearance; (13) the protein content of hydrated amino acid solutions; (14) protein requirements in special situations, including protein-catabolic critical illness; (15) amino acid supplements and additives, including monosodium glutamate and glutamine; and (16) a perspective on the future of protein and amino acid nutrition research. In addition to providing practical information, this tutorial aims to demonstrate the importance of rigorous physiologic reasoning, stimulate intellectual curiosity, and encourage fresh ideas in this dynamic area of human nutrition. In general, references are provided only for topics that are not well covered in modern textbooks. (JPEN J Parenter Enteral Nutr. XXXX;xx:xx-xx)

Keywords

amino acids; dietary proteins; nutrition support; nutrition requirements; nutrition physiologic phenomena; protein; protein requirement

What Are Proteins, and How Are They Evaluated in the Body?

Every protein in the body is synthesized from an intracellular pool of 21 alpha-amino acids—including proline (actually an imino acid) and selenocysteine (in selenoproteins)—that are delivered to the ribosomes bound to specific transfer RNAs. Protein synthesis involves the formation of peptide bonds, in which the amine group on the alpha-carbon (C-2) of one amino acid condenses with a carboxyl group of another amino acid, releasing a molecule of water. The structural diversity of proteins presents a challenge to their measurement. In nutrition, we exploit the fact that the metabolically active proteins in the human body, as well as most dietary proteins, are 16% nitrogen (N) by weight. It has been common practice for >100 years to calculate the amount of protein in a purified protein sample by measuring its N content and multiplying the resulting value by 6.25. The amount of protein in a sample of fat-free muscle or liver—tissues that normally consist almost entirely of hydrated protein—can be determined this way or, in traditional biochemistry, by dehydrating the sample and weighing it to obtain its “dry weight.” The body’s mass of protein-rich cells (muscle, liver, intestines, kidneys, and other organs) is known as the body cell mass (BCM). The BCM is normally hydrated to the extent of approximately 4 g of water : 1 g of protein. By measuring the rate at which dietary N is entering and leaving the body and calculating the difference—N balance—we can infer the rate at which the BCM is increasing or decreasing: multiply N balance (g/d) × 6.25 × 5.

Approximately half the protein in the body is structural and metabolically inactive. This tutorial focuses on the 50% of body protein represented by the BCM: the mass of soluble intracellular proteins that generate and regulate metabolism and enable movement. The BCM makes up approximately 50% of the weight of a healthy young man and approximately 40% of the weight of a healthy young woman. The BCM of a 70-kg man contains approximately 1.12 kg of N: 70 × 0.5 × 0.16 × 0.2. Skeletal muscle makes up 80%–85% of the BCM of a normal, idealized young adult. The remaining 15%–20% consists of the fat-free cells of the organs, plasma proteins,

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blood cells, and immunocytes. This small but metabolically
dynamic compartment of the BCM is called the central protein
compartment, whereas skeletal muscle is called the peripheral
protein compartment.

Why Is Protein an Essential Nutrient?
Approximately 10%–15% of the energy in the diet of wealthy
societies is derived from protein. Humans do not need protein
as a fuel but to increase their BCM during growth, recovery, or
adaptation, and maintain it under steady-state conditions.
Many individual amino acids play important roles as substrates
or precursors of small molecules important in metabolism and
neurotransmission, including

- arginine (precursor of nitric oxide),
- carnitine (synthesized from lysine and methionine),
- creatine (synthesized from arginine, glycine, and
  methionine),
- glutamate,
- gamma-aminobutyric acid (synthesized from
  glutamate),
- glutathione (synthesized from glycine, glutamate, and
cysteine),
- glycine,
- histamine (synthesized from histidine),
- phenylalanine (precursor of catecholamines and thyroid
  hormone),
- taurine (synthesized from cysteine), and
- tryptophan (precursor of 5-hydroxytryptamine and
  niacin).

The amounts of amino acids used for these functions are much
less than the amounts required to maintain the BCM.
Definitions vary somewhat, but in general 9 of the 21 amino
acids used in protein synthesis are termed essential because
they cannot be synthesized in the body and hence are required
in the diet (histidine, isoleucine, leucine, lysine, methionine,
phenylalanine, threonine, tryptophan, and valine). Arginine can
be synthesized but not always sufficiently, and it is considered
conditionally essential. Another 8 amino acids (alanine, aspara-
gine, aspartate, glutamate, glutamine, glycine, proline, and serine)
are nonessential because they are readily synthesized from
widely available carbohydrate molecules and amine groups
provided by the body’s large and rapidly interconverting pool
of free nonessential amino acids (NEAAs)—predominantly,
 glutamine, glutamate, and alanine. The specific contribution of
each NEAA to a nutritional mixture is less important than the
total amount of nonessential N that it provides; thus, amino acid
mixtures used in parenteral nutrition compensate for their lack
of glutamine (and sometimes glutamate or aspartate) by includ-
ing large amounts of glycine and other NEAAs.¹

There are 3 exceptional NEAAs: cysteine, selenocysteine,
and tyrosine. Cysteine and tyrosine are synthesized only from
methionine and phenylalanine, respectively. As long as the
diet contains sufficient amounts of methionine or phenylala-
nine and a person is in a state of near-metabolic homeostasis,
cysteine or tyrosine deficiencies should not occur, because
these amino acids are on the obligatory catabolic pathways of
their corresponding essential amino acid precursors. Thus, in
principle, metabolic steady state with regard to methionine
(methionine intake = methionine catabolism) ought to guaran-
tee the body enough cysteine even when the diet lacks it, since
under steady-state conditions the rate of methionine intake
equals the rate of cysteine synthesis. The composition of the
amino acid mixtures used in parenteral nutrition is somewhat
variable. All of them lack cysteine because it is unstable in
solution; one product contains N-acetylcysteine instead.² The
activity of a non-rate-limiting enzyme on methionine’s cata-
bolic pathway, cystathionase, is reduced in premature infants.
This observation and other supporting evidence—including
hypercystathioninemia, an indicator that metabolic clearance
of cystathionine is reduced—suggest that cysteine could be an
essential amino acid in this situation.³ Definite evidence that
this temporary metabolic blockade is clinically important—
for example, a several-fold increase of urinary cystathionine
excretion unresponsive to therapy with vitamin B₆, the
enzyme’s cofactor—has not been obtained, however,⁴⁻⁶ nor
have confirmatory findings emerged in small metabolic stud-
ies.⁶⁻⁷ Physicians in some neonatal intensive care units (ICUs)
nevertheless add cysteine hydrochloride to parenteral amino
acid mixtures just prior to infusion.¹ The diet is essentially
devoid of selenocysteine, which therefore must be synthesized
in the body in either of 2 ways: catabolism of dietary sele-
nothionine or enzymatic replacement of the sulphur atom in
cysteine by an atom of selenium derived from inorganic
selenite.

The amount of tyrosine in amino acid mixtures is limited
by its poor aqueous solubility. One product uses the water-
soluble tyrosine derivative, N-acetylated tyrosine (NAT),
instead of tyrosine,² but this strategy for increasing tyrosine
provision comes with a mitigating disadvantage—namely,
that humans de-acetylate NAT only slowly and their renal
tubes reabsorb it less efficiently than tyrosine.⁸ One could
speculate that loss of NAT into the urine could become a prob-
lem in diseases associated with aminoaciduria, such as critical
illness, in which a 5-fold increase in urinary-free tyrosine
excretion has been reported.⁹ Tyrosine deficiency seems
unlikely, however, because as long as (1) phenylalanine pro-
vision is sufficient, (2) the patient is in neutral or negative N
balance, and (3) phenylalanine conversion to tyrosine is unim-
peded, the requirement for tyrosine ought to be met by phenyl-
alanine alone.

Ultimately, the catabolic pathways of all amino acids
(including methionine and phenylalanine) flow into the NEAA
pool, which shuttles amino groups among the liver, kidneys,
intestines, and muscles. The NEAA pool serves as a dynamic
reservoir of amines which the liver draws from to insert
glutamate-derived ammonium into the urea synthesis pathway, via the enzyme glutamate dehydrogenase.

N leaves the body in the urine (mostly in urea but also in ammonium and creatinine, as well as in uric acid, protein, and free amino acids but in tiny amounts), in the feces, from the skin (sweat and skin desquamation), and by other miscellaneous routes (hair, nails, secretions, tooth brushing). The final 2 routes are very minor and very difficult to quantify. Modern investigators rely on the results of classic experiments in which dermal and miscellaneous N losses were meticulously measured and reported to amount to ~0.5 g/d for an adult of average weight.10 Sweat contains nontrivial amounts of urea, and loss of body N by this route increases in proportion to the amount that a person sweats. A comprehensive review concluded that the daily total of dermal and miscellaneous N loss may be assumed to be 5 mg/kg for people living in temperate regions and 11 mg/kg for people living in tropical regions,11 for an overall average of 8 mg/kg.12 A healthy 70-kg person eating 100 g/d of protein (16 g of N) will typically excrete 13.5–14.0 g of urinary N, divided among urea (12.5 g), ammonia (1.0 g), creatinine (0.5 g), and uric acid (0.15 g). Basic amino acid absorption and retention.

Obligatory N indicates the healthy body’s maximum capacity to recycle its endogenous proteins, hence minimizing their catabolism. Conditions that impair adaptation, such as critical illness24 and type I diabetes mellitus,25 are associated with increased obligatory N loss, and this implies that these conditions increase the minimum PR.26,27 The minimum dietary PR cannot be less than obligatory N, and it is unlikely to be more than an order of magnitude greater.23 The minimum dietary PR exceeds obligatory N because more efficient recycling of endogenous amino acids is only one component of the adaption to reduced protein intake. The other adaptive component is digestion, absorption, and conservation of dietary amino acids. It has turned out in practice to be very difficult to determine the maximum efficiency of dietary amino acid absorption and retention.

Our current formal estimate of the minimum dietary PR is based on short-term N balance experiments in which healthy volunteers were fed diets that were adequate with regard to every nutrient (especially energy28,29) but in which the amount of high-quality protein was varied. The lowest daily protein intake compatible with zero N balance was defined as the minimum PR. For healthy adults, the average minimum high-quality protein was ~0.65 g/kg body weight per day, with wide interindividual variation. To account for interindividual variability, an amount of protein equal to 2 standard deviations is added to the average minimum requirement to calculate the “safe” or “recommended” protein intake. The recommended daily dietary allowance of high-quality protein is ~0.80 g/kg normal body weight.

N Balance Measurement in Clinical Practice

N balance determinations are useful in clinical care and especially so in critical illness. The rate at which BCM is being lost identifies patients who are experiencing severe protein catabolism, quantifies its extent, and enables the treating
team to assess the effectiveness of the nutrition support regimen that it is using to mitigate the catabolic process. In principle, N balance determinations sufficiently accurate for these purposes are practical in an ICU, for the patient’s protein or amino acid intake is easily quantified and one-on-one nursing makes complete and accurate urine collections feasible. In practice, fecal N is impossible to measure (and increasingly difficult to measure even in research settings), and urinary total N analysis is unavailable in most ICUs. Rather than determining the sum of N in urinary urea, ammonium, and creatinine, critical care specialists have devised formulas to extrapolate total N loss solely from knowledge of urinary urea excretion. Perhaps the best-known formula estimates that total N loss = grams of N in urinary urea / 0.85 + 2 g. When serum urea concentrations increase or decrease considerably, the amount of urea that accumulated (or was lost from) the body during the balance period can be calculated by assuming that urea distributes throughout total body water (50%–60% of body weight). N loss from small exudates is slight, but fluids drained from an open abdomen contain ~2 g of N per liter. A very readable, detailed, and practical review of N balance methodology in clinical practice is available and strongly recommended to all students.

**Is the Minimum PR the Optimum PR?**

A diet is deficient in protein when (1) it is adequate in all other nutrients and (2) it supplies an insufficient amount of protein for the body to remain in zero N balance despite maximum physiologic adaptation. Is protein biology really that simple? Perhaps the process of minimizing endogenous amino acid catabolism incurs a biological disadvantage of some kind. The minimum requirement for vitamin C was long considered to be the lowest rate of consumption that prevents scurvy—as little as 10 mg/d—but as understanding of vitamin C’s metabolism and physiologic roles increased, expert perspective shifted (although not without controversy) to the view that optimal nutrition requires much more vitamin C than required to prevent the classic disease of end-stage vitamin C deficiency. The current recommended dietary allowance for vitamin C is 90 mg. As proved to be the case with vitamin C, protein intakes greater than the minimum amount required to preserve the BCM could serve other important biological functions. This has long been an important topic of speculation.

Different kinds of protein, such as ones of animal or vegetable origin (and their associated food matrices), could be more or less beneficial than others with respect to bone health or cancer, cardiovascular, and renal risk, either in healthy people or in people with certain chronic diseases. This important but complex area of epidemiologic and clinical investigation goes beyond the scope of this tutorial.

**Physiologic and Pathologic Adaptations to Surfeit and Deficient Protein Intakes**

Essential amino acids are toxic in excess, so the body must constantly be able to match amino acid catabolism to the rate of amino acid intake for all intakes at and above the minimum requirement. Transamination and oxidation rates of amino acid catabolic enzymes increase linearly with increasing substrate concentration within the physiologic range. Surfeit amino acid consumption increases the size of free amino acid pools, automatically increasing the amino acid catabolic rate. Whenever the rate of dietary protein intake is at least adequate, the body eliminates amino acids at an equal rate, establishing a metabolic agenda of zero net amino acid retention. When protein intake falls below the requirement level, the metabolic agenda changes from zero-efficiency amino acid disposal to one of efficient retention by (1) more efficient conservation of dietary amino acids and (2) some combination of increased protein synthesis and decreased endogenous protein breakdown—adjustments that limit the size of free amino acid pools (especially in the fed state) and minimize “overflow” catabolism. Amino acid catabolism may be further reduced by adaptive decreases in the amounts and specific catalytic activities of key amino acid catabolic enzymes.

Adaptations of this kind require time to be fully entrained, as suggested by the observation that a certain amount of time is required for amino acid oxidation and resulting N excretion to adjust to a new steady-state rate immediately following an increase or reduction of protein intake.

The term adaptation (more explicitly, normal adaptation) has been proposed to describe normal homeostatic adjustments to variations in protein intake that occur at or above the minimum requirement level and accommodation (or pathologic adaptation) to describe metabolic changes that allow the body to maintain or restore N equilibrium but only by means of an important sacrifice of BCM and physiological compromise, with adverse health implications. People suffering from protein-energy malnutrition (starvation disease) demonstrate accommodation. These chronically starved people commonly reestablish zero N balance despite their continuing inadequate protein and energy intake, at the cost of muscle atrophy, a reduced metabolic rate, and related disabilities. In less extreme situations, the criteria for deciding which specific metabolic adjustments represent “adaptation” and which represent “accommodation” are debated.

**Is Too Much Protein Dangerous?**

Our hunter-gatherer ancestors are estimated to have consumed ~250 g/d of protein, so it is not surprising that high rates of protein consumption become toxic only in the presence of important liver disease (or inadequate perfusion), renal insufficiency, and inborn errors of urea synthesis or amino acid catabolism.
been determined for protein intake. These considerations have no bearing on whether protein intakes greater than the minimum requirement are beneficial, nor do they rule out the theoretical possibility of subtle long-term adverse effects of various kinds.

**Body Composition and the PR**

The human PR is said to be directly proportional to body weight. In mathematical terms, a healthy adult’s daily minimum PR = PR₀ + 0.66W in g/d, where W is body weight in kilograms and PR₀ is a constant that is assumed to be zero. There is no doubt that body weight is a crucial predictor of the PR, but it is very unlikely to be the best predictor. Dietary protein is required to maintain the BCM, so a requirement predictor based on BCM—or a more easily measured but less ideal surrogate, such as lean body mass or fat-free mass—would almost certainly be more accurate and precise than body weight. For example, imagine a healthy young man (BCM = 50% of body weight) who works out and slims down until his BCM now represents 60% of his body weight. It is logical to predict that this person’s minimum PR will increase by 20% from 0.66 g/kg to 0.79 g/kg. Current reviews of PRs in special situations (eg, athletic training, obesity, and old age) could pay more attention to this obvious principle.

Returning to the PR equation, PR = PR₀ + 0.66W, it is worth pointing out that there is no physiologic basis underlying the assumption that PR and W (or BCM) are directly proportional. The peripheral and central compartments of the BCM have very different metabolic activities, and their masses tend not to increase and decrease proportionately. These considerations predict a nonlinear relationship between PR and BCM. Even if the relationship is adequately linear, which it may well be in the midrange of body weights, there is no physiologic justification for the universal assumption that PR₀ (the so-called y-intercept) = 0. It is quite likely that rather than being zero, PR₀ is actually a large number, just as it is in all equations used to predict energy expenditure, such as the Harris-Benedict equations. Unlike the situation with energy expenditure predictors, we simply do not have enough data to characterize the relationship between PR and W (or BCM) except in the crudest way.

**Labile Protein**

Figure 1, reproduced from a classic review of protein nutrition, illustrates that as soon as a person’s N intake was reduced from 16 to 5 g/d, their N balance became strongly negative. Urinary N excretion decreased over the subsequent 4 or 5 days to a level that reestablished near-zero N balance. Increased N intake stimulated a transiently positive cumulative N balance of the same magnitude, as indicated by the equal hatched areas under the 2 N excretion curves.

![Figure 1](image)

The body protein lost and regained in this process is believed to represent the dissolution and redeposition of a rapidly turning over protein pool in the body, called *labile protein*. Labile protein may reside in the liver and splanchnic bed. It is presumed to consist, in part, of amino acid–metabolizing enzymes whose activity and mass decrease and increase in response to a sustained reduction or increase in protein intake.

Labile protein has long intrigued protein nutrition scientists; we still do not know exactly what it is. One expert speculated that the gradual reduction in urinary N shown in Figure 1 is fully explained by delayed passive movement of urea from body water into the urine rather than a delayed reduction in the rate of amino acid catabolism. Let’s examine the data. The hatched area in Figure 1 represents a loss to the body of ~12 g of N, or 12 × 6.25 = 75 g of labile protein. (Direct body measurement indicates a similar figure, and a roughly similar value of ~20 g of N can be calculated from the data in a study that determined the rate of adaptation to a protein-deficient diet.) The switch from a high-protein to a low-protein diet reduces the serum urea concentration by ~1.75 mmol/L, so if we assume that the person in Figure 1 weighed 70 kg and their body was 60% water, the 1.75-mmol/L decrease in serum urea concentration that resulted from the change to the low-protein diet implies that 1.75 mmol/L × 0.6 × 70 = 73.5 mmol of urea = 4.41 g of urea = 2.1 g of urea.
How Accurate Is N Balance?

Our current estimate of the minimum PR is based on N balance data. The biological interpretation of N balance is straightforward, but the measurement itself can be problematic. In requirement studies, the most interesting and important value for N balance is zero, but methodological and analytic errors are magnified whenever a small number is calculated as the difference between 2 large ones (N intake – N loss). Furthermore, for methodological and analytic errors are magnified whenever a small number is calculated as the difference between 2 large ones (N intake – N loss). Moreover, for methodological and analytic errors are magnified whenever a small number is calculated as the difference between 2 large ones (N intake – N loss). Furthermore, for methodological and analytic errors are magnified whenever a small number is calculated as the difference between 2 large ones (N intake – N loss).

Unrealistically positive N balances are not uncommonly reported in the research literature. How should we interpret them? Do they indicate that there is a systematic positive error in all the N balance data in the study, or—as one might surmise when the study’s methodology is sound—do the unrealistically positive balances apply only to very high N intakes? Whatever the best answer is for any given study, a recent comprehensive analysis indicates what we should not do: regard the systematically high N balances observed at very high protein intakes as biologically real.

For example, the N balance data upon which the current adult minimum PR is based were recently reanalyzed through a biphasic regression model that gave infinite mathematical weight to the positive N balances associated with surfeit protein intakes. The conclusion of this analysis was that the adult minimum daily PR is 0.91 g/kg, ~50% higher than the current value of 0.66 g/kg. The validity of this conclusion rests on the questionable premise that the infinitely weighted N balance data at surfeit protein intakes are biologically real.


Protein and Amino Acid Turnover

Despite its technical shortcomings, N balance remains the gold standard metabolic measurement in protein nutrition—first, because its technical shortcomings are relatively minor when meticulously carried out and when protein intake is not extremely high and, second, because its biological interpretation is straightforward. The truly serious shortcoming of N balance was pointed out by the 19th-century physiologist Claude Bernard, who remarked that one cannot hope to understand what goes on inside a house just by analyzing what goes in the doors and out of the chimney. N balance cannot inform us about the mechanisms that regulate metabolic adaptation to different diets or the effects of different diseases on protein metabolism. Several models of human amino acid kinetics have been developed to find out what is actually going on “inside the house.” It will suffice here to describe a simple steady-state amino acid turnover model that has been used to estimate human essential amino acid requirements. The model technique involves the continuous administration of a tracer-labeled essential amino acid, such as L-13C-leucine.

When isotopic equilibrium is successfully established, the rate of tracer infusion equals the rate that it is disappearing from the sampling compartment. By measuring the extent to which the tracer amino acid is diluted by its parent unlabeled amino acid (tracee), one can calculate the rate at which tracee molecules must be entering and leaving the system. Thus, the rate of tracee appearance (also called turnover, flux, or Q) = tracer infusion rate / tracer dilution. Tracee appears in the system as a result of endogenous protein breakdown (B) or, in fed-state studies, as B + the rate of tracee administration (I). Tracee disappears from the system at the same rate that it appears, both for deposition in proteins (protein synthesis [S]) and for oxidation,
ing the particular mixtures of free amino acids to which they consume repeated small meals containing an amino acid mixture values by dividing the turnover and oxidation rate of the tracer amino acid by its fractional representation in the BCM. Thus, leucine makes up 8% of the weight of the BCM, so whole body protein turnover can be estimated by dividing leucine turnover (expressed as mg/time) by 0.08. If the model is valid, net body protein catabolism calculated from this procedure—the difference between S and B—should equal N balance; steady-state oxidation (E) should correspond to whole body N excretion.70,71 This model of whole body protein turnover reduces the body’s complex pattern of protein turnover, synthesis, breakdown, and amino acid catabolism to a single compartment with single values of Q, B, S, and E, even though we know that protein kinetics differ considerably in different tissues. Sophisticated organ-specific (usually muscle) turnover models have been developed and proven very useful, but their details go beyond the scope of this tutorial.72

Steady-state amino acid turnover models require isotopic steady state and a constant size of the tracee pool, the evidence for which is an unchanging plasma concentration of the tracee amino acid. Even when these 2 requirement are met, the data generated by a turnover study indicate only what is happening “right now”—during this particular moment of isotopic and tracee steady state. Isotopic steady state neither implies nor guarantees metabolic steady state. If an individual is not in metabolic steady state on the particular day that their protein turnover is being measured, it is inappropriate—but, unfortunately, not uncommon—to interpret the experimental results as definitively characterizing the ultimate effect of the particular diet or treatment being administered. It is important to appreciate that quite different results might be obtained if one allowed 1, 2, or 4 more days of continued diet or treatment before making the measurements.

**Amino Acid Turnover Models and the Estimation of Essential Amino Acid Requirements**

Two amino acid turnover models have been developed to estimate essential amino acid requirements. The first model—the direct amino acid oxidation (DAAO) technique—involves adapting research volunteers for several days to diets that are adequate in all nutrients except the essential amino acid whose requirement is being investigated. On different study days, the volunteers consume repeated small meals containing the particular mixtures of free amino acids to which they have become adapted. When the diet is low in the test amino acid (eg, lysine), the body adaptively reduces lysine catabolism to remain in zero lysine balance. The attempt fails when the amount of lysine in the diet is less than the body’s maximum ability to reduce lysine catabolism. As the amount of lysine in the different test diets increases to match, then exceed, the minimum requirement, lysine oxidation rates progressively increase to maintain zero lysine balance. The lowest lysine intake at which lysine oxidation begins to increase (and lysine balance becomes zero) is the minimum lysine requirement. A different model—the indicator amino acid oxidation (IAAO) technique—avoids the nontrivial problem of accurately calculating amino acid balances near zero (the same problem that occurs with N balance), as well as the theoretical possibility that tracer/tracee distortions could be introduced when tracer and the tracee amino acid whose intake is being varied are the same molecule. As with the DAAO technique, on each tracer study day volunteers consume repeated small meals containing an amino acid mixture that contains constant adequate amounts of all amino acids except the amino acid whose requirement is being investigated (eg, lysine), which is provided in different amounts on different study days. In this method, the tracer is not lysine but a different essential amino acid: the indicator amino acid. When lysine provision is inadequate, protein synthesis is unable to proceed efficiently, so the rate of oxidation of all amino acids other than lysine increases inappropriately in relation to the rate that they are being administered. As the amount of lysine in different test meals increases to match, then exceed, the minimum requirement, all of these amino acids are more efficiently deposited in protein, so their oxidation rate decreases to match the rate at which they are being administered. The lowest rate of lysine provision compatible with efficient utilization of the dietary mixture is defined as its minimum requirement. In this model, the oxidation rate of the indicator amino acid is a surrogate for total amino acid oxidation. IAAO provides exactly the same biological information that total N excretion provides over a longer time scale.

Some of the assumptions of the IAAO technique are debated.71,74 Here I focus on the assertion that this technique accurately identifies the minimum essential amino acid requirement after only a handful of hours of exposure to the test diet; that is, there is no need to allow time for adaptation.58,66 This assertion explicitly contradicts the widely accepted notion that the main determinant of a nutrient’s minimum requirement is the body’s ability to adapt to low intakes, a process that requires at least a few days.75 One review asserts that several days of adaptation are required when the DAAO technique is used but not when the IAAO technique is used,59 but this does not make sense. If adaptation is a real phenomenon, how can allowance for it be required for one model but not another one? In general, whenever a paradigm-busting scientific claim is made, it is normal for the claimants to explain
and defend their claim in the context of the existing paradigm, then proceed to demonstrate its validity using a variety of different methods.

Yet, some data do support the claim that the IAAO model is exempt from adaptation—although in my opinion, inadequately, in view of how radical it is. Is it possible that adaptation occurs in some situations but not others? Humans evolved as hunter-gatherer omnivores and hence would not have been subject to evolutionary pressure to adapt to individual amino acid deficiencies. Perhaps humans are simply not biologically equipped to adapt to selectively lysine-deficient diets. Such a notion seems to be suggested by the severe anorexia, fatigue, and irritability that abruptly develop immediately after a severely essential amino acid–deficient diet is introduced. This clinical picture of metabolic disarray is quite unlike the coordinated metabolic response to fasting or general protein deprivation. If it really is the case that humans are physiologically incapable of adapting to selective essential amino acid deficiencies, then the IAAO proponents are right: there really is no need to allow time for adaptation before characterizing a person’s response to different rates of administration, no matter what model is used.

It would be easy to test this hypothesis without having to grapple with the problems of drawing precise numeric conclusions from data generated by mathematical models with their debatable premises and quantitative assumptions. One could simply adapt normal volunteers to a conventional diet, then feed them a comparable but indisputably lysine-deficient diet for 7 days while tracking their plasma lysine concentrations and urinary N excretion, knowing as we do that urinary N excretion provides exactly the same information as IAAO over a longer time scale. N balance will turn negative immediately after the diet becomes lysine deficient. If humans have mechanisms to adapt to selective lysine deficiency, then as lysine’s plasma and tissue concentrations decrease over time, the adaptive mechanism will kick in to reduce lysine catabolism, making more lysine available for protein synthesis and allowing N balance to climb back toward zero. If, however, adaptation is not an option available to humans, their N balance will promptly turn negative when the diet becomes lysine deficient and remain just as negative for the following 7 days or longer.

How to Evaluate a Protein Turnover Article

The foregoing description of 2 steady-state amino acid turnover models should equip students to comprehend almost any amino acid turnover model, even though some of them are very sophisticated. It is useful to go down a mental checklist when reading any amino acid turnover article.

Is the Model Comprehensible?

Every turnover model is an oversimplification of biology. The authors of any published article should explain the simplifying assumptions that went into its development, justify them, and explain how the simplifying assumptions qualify the conclusions of the study. Every well-written research article should be comprehensible to an intelligent reader.

Is the Model Appropriate for the Specific Question or Hypothesis Being Tested?

A very simple model may suffice to answer a basic biological question, but subtle, clinically oriented questions can be problematic. For example, the experimental conditions that have to be imposed to create isotopic steady state are often highly atypical of normal nutrition and metabolism. Steady state requires continuous rather than episodic nutrient provision and, in fed-state studies, the provision of artificial mixtures of free amino acids rather than whole proteins. It is always prudent to enquire how confidently the results of such a laboratory experiment can be extrapolated to the normal physiologic situation.

Were the Research Volunteers Fully Adapted/Habituated to the Experimental Diet or Treatment?

A turnover study that documents isotopic steady state may still be questionable if the experimental volunteers were metabolically unstable or in a changing metabolic state on the day that the study was carried out. Plasma amino acid concentrations and other relevant substrate or hormonal levels should all be unchanging. If they are changing, the patient cannot be in metabolic steady state, and any conclusions drawn from the data are doubtful. If a research subject has not fully adapted to an experimental diet or treatment—a process that appears to require at least 3 or 4 days—any conclusions about its effects must be highly qualified.

Amino Acid Turnover and Body Composition

As explained with regard to the relationship between body composition and PRs, a problem arises when authors report protein turnover values per unit of body weight (or even fat-free mass) in situations in which body composition is changing or deviates importantly from normal. Protein turnover proceeds at different rates in the different compartments of the BCM, which are commonly depleted and repleted to differing degrees in starvation and anabolism. For example, reports that chronic protein-energy starvation leads to an abnormally high rate of whole body protein turnover (expressed either per kg of body weight or fat-free mass) are likely simply due to the disproportionately greater loss of slowly turning over skeletal
muscle protein mass than rapidly turning over central protein mass that occurs in this situation.\textsuperscript{24}

**Turnover Versus Clearance**

Turnover (also called appearance or disappearance rate or flux) is the rate at which a substrate transits into, through, and out of a system under steady-state conditions. Clearance is defined as turnover / substrate concentration in the compartment of interest; its units are volume/time. Subnormal clearance of a solute indicates that a subnormal volume of solvent (eg, extracellular fluid) is being “cleared” of a given amount of solute per unit time. The underlying concept is that conditions that lower substrate clearance are ones that require an increased substrate concentration to maintain a constant turnover rate. Thus, people with stable non–end stage renal failure are in zero N balance, excreting urea at a rate commensurate with the amount of protein they eat. This feat is accomplished, in the face of a low functional renal mass, by increasing the amount of urea in each milliliter of glomerular filtrate. The indicators of this process are (1) an increased plasma urea concentration and (2) reduced urea clearance, measured as urea excretion rate / plasma urea concentration.

The rate at which metabolites flow down a particular biochemical pathway does not, by itself, allow a definite conclusion about the presence or absence of partial blocks on the pathway. In one study,\textsuperscript{79} investigators set about to determine whether the hyperhomocysteinemia of chronic renal failure is caused by impaired remethylation of homocysteine back to methionine—a reaction that recycles homocysteine back to the top of methionine’s catabolic pathway—or by impaired catabolism to cysteine via the transsulfuration (TS) pathway. Upon observing normal TS rates of patients with hemodialysis-dependent end-stage renal failure, the authors concluded that the hyperhomocysteinemia of end-stage renal disease is not due to impaired TS but rather to impaired remethylation. This conclusion is wrong. The TS rate is the rate at which methionine and homocysteine are eliminated from the body. Under steady-state conditions, the TS rate must equal methionine intake. Since people with stable chronic renal failure are in metabolic equilibrium, they catabolize methionine and eliminate urea at rates commensurate with their rate of consumption of methionine and protein. The observation that the TS rate of such persons was normal simply means that, when they were studied, their methionine intake was normal. The metabolic block responsible for hyperhomocysteinemia in renal failure is indeed on the TS pathway. Its signal is hyperhomocysteinemia and reduced homocysteine clearance. Renal failure reduces the amount (and/or catalytic activity) of the rate-limiting enzyme of homocysteine catabolism: cystathionine-beta-synthase. As a consequence, the enzyme needs a higher concentration of its substrate, homocysteine, to process homocysteine at a rate commensurate with its rate of arrival.\textsuperscript{80}

**The Molecular Weight of a Mixture of Free Amino Acids Is Greater Than the Molecular Weight of the Protein They Create**

Peptide bond formation releases a molecule of water, so any mixture of free amino acids necessarily has a lower N density that the protein that it creates.\textsuperscript{81} People who fail to appreciate this obvious biochemical fact will overestimate the amount of protein substrate in a parenteral nutrition solution (eg, 100 g of mixed amino acids provides 83 g of actual protein substrate\textsuperscript{81}), and they will overestimate the minimum requirement for protein-bound essential amino acids. The error is relatively small for individual essential amino acids such as lysine (12% error: formula 146, molecular weight in protein 128) or leucine (14% error), but it becomes decidedly nontrivial when an entire mixture of free amino acids is used as a surrogate for dietary protein and the total nominal weight of the amino acids in the mixture is erroneously assumed to be equivalent to the same weight of formed protein.\textsuperscript{68,74,82,83} Unless accounted for, this mathematical error will lead to an important overestimation of the minimum PR.

**Do Protein Calories Count?**

Confusion sometimes arises, even in published articles, about the amount of energy provided by dietary protein. Some people find it difficult to understand how amino acids can be taken up into the BCM and simultaneously oxidized as an energy source. The apparent paradox is resolved by remembering the steady-state hypothesis. A person in metabolic homeostasis is in zero N balance: amino acid oxidation equals amino acid intake. For every amino acid that the BCM takes up, it releases an equivalent amino acid in the course of normal protein turnover. Thus protein simultaneously provides fuel and maintains the BCM. It is certainly true that someone who is in positive N balance will oxidize amino acids at lower rate than the rate of intake. However, even under strongly anabolic conditions, N balance is seldom more positive than 5 g/d, which is equivalent to only $5 \times 6.25 \times 4 \text{ kcal/g} = 125 \text{ kcal/d}$ rendered unavailable for oxidation. By contrast, a critically ill person may excrete 15 g/d of N even when exogenous protein provision is nil. Such a person “receives” $15 \times 6.25 \times 4 = 375 \text{ kcal of fuel energy per day}$ from burning up their muscle mass. This is not an insignificant amount of energy! This source of energy provision is never considered in clinical practice; perhaps it should be. It is worth remembering that formed protein provides 4 kcal/g, whereas hydrated amino acids provide only 3.4 kcal/g.\textsuperscript{81}

**Are the Article’s Conclusions in Keeping With the Limitations of the Data?**

One should be cautious when interpreting claims of the nutrition benefits of a specific diet or supplement on the basis of...
biodendritic cell pertaining only to an isolated event, such as the acute rate of muscle protein synthesis. How can we be confident that the short-term effect detected in a single tissue is relevant to the whole body in normal life? A certain supplement or diet could acutely stimulate muscle protein synthesis under specific experimental conditions—and the observation may well provide important insight into muscle physiology—but any conclusion about its overall health implications should be tempered by skepticism. When a person is in metabolic homeostasis, any artificial increase in muscle protein synthesis would be expected to stimulate an equal, compensatory increase in muscle proteolysis, either simultaneously or shortly afterward, with the result that nothing changes. If proteolysis is not measured, how can we conclude that the treatment increases muscle mass? Alternatively, a treatment may inhibit muscle protein breakdown without reducing muscle protein synthesis. Such a treatment could usefully increase total muscle mass or prevent muscle atrophy. But what if the consequence of this effect is to trap free amino acids in muscle, starving the central protein compartment of the amino acids that it needs?

Special Situations Increase Uncertainty and Require Rigorous Physiologic Reasoning

The minimum requirements for protein and all other essential nutrients are established (1) with specific reference to healthy individuals and (2) with the explicit proviso that the requirement that is set for any nutrient assumes that the minimum requirements of all the other ones are being fully met. Regrettably, many clinicians are unaware of these qualifications and assume that disease, concurrent deficiencies of other nutrients, or other abnormal physiologic states have no effect on nutrient requirements. They are wrong. Human physiology is so dynamic and malleable that the notion that every nutrient has a single minimum requirement value that suits every situation—emotionally comforting though it is, and as scientifically important as it is to establish a benchmark—is naive and foolish. Every published requirement value should be regarded skeptically and as a smudge rather than a fine point.

One can use physiologic principles to extrapolate a sick person’s nutrition requirements from the best available data, starting from the benchmark provided by the minimum requirement of a healthy person. The importance and, indeed, essentiality of sound physiologic reasoning in situations like this can be illustrated by considering a topic of great current interest: nutrition—rigorous mitigation of bias and a commitment to rational reasoning within a conceptually sound physiologic paradigm—is as important in the accumulation and interpretation of preclinical and physiologic-metabolic evidence as it is in the design and interpretation of randomized clinical trials. Unbiased interpretation of physiologic-metabolic evidence requires physiologic literacy and good judgment; it is intellectually demanding, time-consuming work, and it typically yields only provisional conclusions. But scientific integrity requires it. Nutrition investigators have a duty to avoid cherry-picking physiologic and metabolic evidence as important as their duty to avoid cherry-picking clinical trial data.

PRs in Early Childhood and Growth

The reliable estimation of the protein and amino acid requirements of neonates and children is extremely important but very complicated and very difficult; this topic merits a tutorial review of its own. The same physiologic principles explained in this tutorial apply, but clinical studies are much more difficult to carry out, and the estimates arrived at require sophisticated inference from many sources, including observational and biological information, aided by target outcomes such as linear growth and the rate of accretion of body mass.
Amino Acids as Supplements and Additives

Many individual free amino acids are available as supplements, including arginine, citrulline, glutamate, glutamine, leucine, lysine, S-adenosylmethionine, and tryptophan. When one considers the biological plausibility or potential toxicity of an amino acid supplement, it is useful to compare the dose on the product label with the amount that is consumed in a typical diet, as well as its rate of body turnover, which typically greatly exceeds the rate of intake. Many widely marketed supplements contain utterly insignificant amounts of amino acids. Others could have unique biological effects when taken in large boluses apart from the other amino acids present in dietary protein. Glutamic acid’s sodium salt, monosodium glutamate (MSG), is widely used by the food industry as a tasty food additive. Some people are convinced that MSG causes alarming or dangerous symptoms in susceptible people: the MSG syndrome. At least one U.S. government website claims that the MSG syndrome is real, but many scientists discount it, partly because the clinical syndrome is very poorly documented and partly because the amount of MSG that is eaten in a meal is trivial in comparison to the amount of glutamate that is consumed in protein-bound form in the same meal and the even vaster amounts that are synthesized and metabolized within the body every day. The recent discovery of glutamate “taste” receptors throughout the gastrointestinal tract could shed new light on the MSG syndrome.

The modern ICU has become a clinical laboratory for studying the therapeutic effects of many individual amino acids, including arginine, sulfur amino acids as glutathione precursors, and glutamine. One of the problems with evaluating the therapeutic potential of glutamine is its rapid interconversion with other amino acids in the NEAA pool, which receives amine groups from the catabolism of all amino acids and hence is richly replenished by normal protein turnover and amino acid catabolism. Normalized to a body weight of 70 kg, glutamine appears in the circulation at a rate of 3 g/h under basal conditions and approximately 3 times faster than this in the early phase of acute catabolic disease.

By comparison, the doses of glutamine used in clinical trials have averaged ~0.3 g/kg/d (0.9 g/h for a 70-kg patient). Glutamine infusion rates of 0.2–0.35 g/kg/d are considered “high doses.” When such small amounts of glutamine are infused in relation to its many-fold-greater endogenous turnover rate, there is a considerable risk of obtaining indeterminate results from clinical trials. The rate of endogenous glutamine release will vary greatly among patients as determined by their existing muscle mass and the intensity of their catabolic state, as well as by the concurrent rate of administration of glutamine’s immediate precursor, protein.

Despite the strong physiologic evidence that critical illness increases the PR, most patients in modern ICUs are remarkably protein deprived. One author even speculated that administering large doses of any single amino acid (eg, glutamine) could create distortions and toxicities in patients who are as severely protein deficient as most modern ICU patients. Such a toxic effect seems unlikely, however, given the extremely low rates at which glutamine has been administered in clinical trials in comparison to its many-fold-greater endogenous turnover rate.

Customized solutions that contain glutamine or arginine can be difficult to obtain, but every ICU has parenteral amino acid mixtures available for immediate use. If infused in sufficient amounts, these amino acid mixtures, despite being glutamine-free, would provide the substrate that the body requires to generate very large amounts of glutamine. How much glutamine could a nutritional mixture of amino acids provide? A 70-kg patient infused 2 g of protein substrate/kg body weight per day—a physiologically plausible dose—would receive 140 g of protein and catabolize essentially all of it, thus making 140 × 0.16 = 22.4 g of N = 22.4/14 = 1.6 mole of N available for glutamine synthesis. This is enough N to synthesize as much as 0.8 moles (glutamine has 2 N atoms) = 0.8 × 116 g/d of glutamine, or 4.8 g/h of glutamine—if the body chose to do so. Thus, simply providing a generous nutritional mixture of amino acids would enable the body to use its own machinery to generate glutamine as much as 5 times faster than the average rate at which glutamine has been administered in published clinical trials. And there are major added benefits: this same mixture of amino acids would provide large amounts of arginine and methionine (the latter for glutathione synthesis) and the supra-additive benefit of stimulating central protein synthesis and protecting the peripheral muscle mass.

The notion that critically ill patients would benefit from suitably generous amino acid provision is hardly new; it has been embedded in critical care practice guidelines for decades. Could high-dose mixed amino acid infusions actually increase protein central synthesis, protect muscle mass, and provide the benefits of an adequate supply of glutamine, arginine, and methionine, the precursor of glutathione? It is highly plausible, but no one knows! More than 2 dozen high-quality clinical trials of glutamine infusion have been published with indeterminate results, but as of the time of this writing, not even one modern high-quality trial of suitably generous mixed amino acid provision (eg, 2.0–2.5 g/kg) has appeared in the literature.

Will We Ever Agree on PRs?

Carpenter’s monograph Protein and Energy: A Study of Changing Ideas in Nutrition is required—and humbling—reading for anyone who is seriously interested in the science of human nutrition. Protein intake recommendations have been earnestly debated for more than a century, but uncertainty persists. The reasons are not difficult to ascertain: the question is biologically complex (it is not always obvious even what question to ask); there are important nutrient-nutrient interactions (especially with energy); the requirement value is universally based on body weight, an unacceptable surrogate
for BCM; there is enormous variability in individual responses to different levels of protein provision (unlike with other nutrients, its genetic basis has not been investigated); the amount of reliable metabolic data is scanty; and the kind of hard functional outcome evidence that is increasingly required to justify public health recommendations is essentially nonexistent. Even if one trusts the N balance technique, the amount of high-quality N balance data that has accumulated over many decades in the effort to identify minimum PRs is dwarfed, by at least an order of magnitude, by the amount of clinical data routinely gathered in any one of the dozens of clinical trials of lipid-lowering and other drugs that appear regularly in the literature. Society plainly has much more interest in identifying new drug products than in identifying the minimum PR.

With the instructive exception of athletic training protocols that incorporate muscle imaging, functional outcomes, and prolonged follow-up periods, our current methods for estimating the minimum PR are short-term and only inferential, since they rely on metabolic markers whose true, long-term physiologic pertinence can be questioned. N balance, acute muscle protein synthesis rates, and amino acid oxidation rates are not clinical outcomes. Sophisticated turnover studies are of undoubted value for elucidating particular aspects of amino acid metabolism and gaining insight into its regulation, but it is asking a lot of them—perhaps too much—to provide accurate specific requirement numbers when the best that they can do, in small handfuls of experimental subjects, is generate metabolic snapshots of metabolic processes that vary widely and mysteriously among individuals and over time and are subject to metabolic adaptations and environmental drivers that are not accounted for in a clinical research laboratory.

Intriguingly, the very kind of hard physiologic outcome studies that would be needed to shift our perspective on human PRs were carried out >100 years ago at Yale University by Russell H. Chittenden. Chittenden’s meticulous long-term studies of the physiologic effects of limiting human protein consumption, as well as other clinical studies carried out in the early 20th century, suggest an average minimum PR close to the current value of ~0.65 g/kg/d. These venerable but high-quality observations suggest that the current minimum requirement value may actually be closer to optimal. Chittenden’s work is in print and available for study but widely ignored. His work was not without flaws, but the chief reason that it was rejected was not lack of quality but rather because his conclusions were distasteful to the circular cultural bias of the time, which held that because healthy people are accustomed to consuming a large amount of protein, a healthy diet ought therefore to include a large amount of protein.

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This tutorial is dedicated to H. N. Munro (1915–1994) and V. R. Young (1937–2004), professeurs extraordinaires in the Department of Nutrition, Massachusetts Institute of Technology.

Glossary

- **Accommodation**: Metabolic changes that allow the body to remain in N equilibrium but only by means of an important sacrifice of body cell mass or other adverse health consequences.
- **Adaptation**: Normal homeostatic adjustments to variations in protein intake at or above the minimum requirement level.
- **Body cell mass**: Mass of metabolically active soluble intracellular proteins that generate and regulate metabolism and enable movement.
- **Clearance**: Volume of a compartment that is completely “cleared” of a particular substrate per unit of time, measured as the turnover (or disposal) rate divided by substrate concentration. The key concept is that conditions that lower substrate clearance are ones that require the body to increase substrate concentration to maintain a constant turnover rate.
- **Half-life**: The amount of time required for half the substrate molecules in a compartment to be replaced by newly entering ones.
- **Labile protein**: A small, rapidly turning over pool of protein that is gained or lost during the adaptation to variations in protein intake and may be involved in adaptation.
- **Minimum protein requirement**: Lowest level of protein intake at which the body is capable of remaining in zero nitrogen balance.
- **Nitrogen balance**: The difference between N intake and N loss; indicator of body protein balance.
- **Nutrition habituation**: Regulated metabolic adjustments to changes in nutrient intake, absorption, or metabolism that occur within the normal adaptive range, hence without health implications.
- **Obligatory nitrogen excretion**: Rate of nitrogen loss after adaptation to a protein-free diet; a measure of the maximum capacity to recycle endogenous proteins, hence minimizing their catabolism.
- **Optimum protein requirement**: A theoretically proposed protein intake that exceeds the minimum necessary to preserve the body cell mass, conferring health benefits through other biological mechanisms.
- **Recommended dietary allowance for protein**: The average minimum protein requirement for a healthy person plus 2 standard deviations to allow for individual variability.
- **Turnover**: The rate at which a substrate enters, passes through, and exits a body compartment under steady-state conditions. Synonyms are flux, appearance rate, disappearance rate, and disposal rate.
- **Turnover time**: The amount of time required for all the substrate molecules in a compartment to be replaced by newly entering ones; turnover time = half-life/0.693.

Statement of Authorship

L. John Hoffer was solely responsible for conceiving and designing this review and for the acquisition, analysis, and interpretation of the data presented in it, its drafting, and all revisions to it. He agrees to be fully accountable for ensuring the integrity and accuracy of the work, and he read and approved the final manuscript.

References


