Defining meal requirements for protein to optimize metabolic roles of amino acids[1–5]

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ABSTRACT

Dietary protein provides essential amino acids (EAAs) for the synthesis of new proteins plus an array of other metabolic functions; many of these functions are sensitive to postprandial plasma and intracellular amino acid concentrations. Recent research has focused on amino acids as metabolic signals that influence the rate of protein synthesis, inflammation responses, mitochondrial activity, and satiety, exerting their influence through signaling systems including mammalian/mechanistic target of rapamycin complex 1 (mTORC1), general control nonrepressed 2 (GCN2), glucagon-like peptide 1 (GLP-1), peptide YY (PYY), serotonin, and insulin. These signals represent meal-based responses to dietary protein. The best characterized of these signals is the leucine-induced activation of mTORC1, which leads to the stimulation of skeletal muscle protein synthesis after ingestion of a meal that contains protein. The response of this metabolic pathway to dietary protein (i.e., meal threshold) declines with advancing age or reduced physical activity. Current dietary recommendations for protein are focused on total daily intake of 0.8 g/kg body weight, but new research suggests daily needs for older adults of ≥1.0 g/kg and identifies anabolic and metabolic benefits to consuming at least 20–30 g protein at a given meal. Resistance exercise appears to increase the efficiency of EAA use for muscle anabolism and to lower the meal threshold for stimulation of protein synthesis. Applying this information to a typical 3-meal-a-day dietary plan results in protein intakes that are well within the guidelines of the Dietary Reference Intakes for acceptable macronutrient intakes. The meal threshold concept for dietary protein emphasizes a need for redistribution of dietary protein for optimum metabolic health. *Am J Clin Nutr* 2015;101(Suppl):1330S–8S.

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INTRODUCTION

Dietary protein intakes needed for optimal long-term health remain controversial. Studies investigating physiologic and metabolic changes during aging (1, 2), weight loss (3, 4), bed rest (5), and treatments for type 2 diabetes (6) or metabolic syndrome (7) reported benefits of diets with protein intakes of 1.2–1.6 g/kg body weight or >20% of energy intake. These intakes are above the Recommended Dietary Allowance (RDA).[6] Contrary to these reports, there are studies that reported no beneficial effects of higher protein (8–10); and the 2010 Dietary Guidelines Advisory Committee report stated, "Protein intake in the United States is more than adequate" and that inadequate protein in the United States is rare (11). These divergent views, at least in part, arise from definitions of protein adequacy based on measures of nitrogen balance compared with evaluation of metabolic roles of individual amino acids.

Metabolic roles for amino acids are diverse and include at least 4 categorical functions or roles: substrates for messenger

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[6] Abbreviations used: BCAA, branched-chain amino acid; BCKDC, branched-chain keto acid dehydrogenase complex; EAA, essential amino acid; EAR, Estimated Average Requirement; GCN2, general control nonrepressed 2; IAAO, indicator amino acid oxidation; IGF, insulin-like growth factor; IRS-1, insulin receptor substrate 1; mRNA, messenger RNA; mTORC1, mammalian/mechanistic target of rapamycin complex 1; PKB, protein kinase B; p70S6K, 70-kDa S6 protein kinase; RDA, Recommended Dietary Allowance; tRNA, transfer RNA; UL, Upper Limit; 4EBP1, 4E binding protein 1.

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RNA (mRNA) translation; initiators of signal transduction and neurotransmission; biosynthesis of other nitrogen-containing compounds such as glutathione, creatine, taurine, carnitine, nitric oxide, serotonin, and thyroxin; and formation of nonnitrogenous compounds for gluconeogenesis, one-carbon methyl reactions, and anaplerotic balance of the tricarboxylic acid cycle. For each of these roles, plasma or intracellular amino acid concentrations, and ultimately dietary intake, affect the amino acid flux through the pathway and physiologic outcome. These metabolic pathways and outcomes that are sensitive to dietary protein intake and relate to recognized indexes of health should be considered in determining optimum dietary goals for protein. This review explores the metabolic consequences of dietary amino acids and positions this information within the context of defining protein needs for adults. New information about optimum meal distribution of protein for muscle health and satiety will be emphasized.

IS THE RDA REALLY THE BEST MEASURE OF DIETARY PROTEIN NEEDS?

Nitrogen balance is the conventional measure of protein needs used in crafting the RDA, and it reflects efficiency of nitrogen retention under conditions of energy balance (12–14). In this context, additional protein intake above that required for attaining nitrogen balance has been viewed as unnecessary or possibly unsafe. The RDA for both men and women (≥19 y old) is 0.80 g high-quality protein per kilogram of body weight per day and is based on the minimum dietary protein required to achieve nitrogen balance. Nitrogen losses reflect the daily requirement to replace essential amino acids (EAAs) lost to degradation pathways and are estimated by collection of nitrogen in urine, stool, breath, skin, and hair and extrapolated to dietary protein (15). For estimation of the RDA, or more specifically, the Estimated Average Requirement (EAR), dietary protein is titrated down to the minimum amount that allows the body to achieve nitrogen balance and uses a monolinear regression to calculate a breakpoint for the EAR (16). This represents an obligatory rate of amino acid degradation; however, the rate of nitrogen loss has no direct relation to other metabolic roles of amino acids. Inherent in the nitrogen balance approach is the assumption that dietary goals for protein intake equate with efficiency of amino acid use for nitrogen-containing molecules only. This singular focus on attaining the lowest possible amino acid oxidation suggests that increases in the intracellular concentrations of amino acids or their keto acid carbon skeletons are unnecessary and perhaps unfavorable.

Although the RDA may represent a minimum amino acid requirement for most healthy individuals, higher intakes of EAAs or indispensible amino acids may impart metabolic benefits, including improved body composition (e.g., maintenance, growth, or function of lean mass), enhanced satiety, increased thermogenesis, or improved glycemic regulation (17), and may aid in recovery after trauma, surgery, or prolonged bed rest (5). Variables related to muscle mass, strength, and metabolic function have been proposed as other relevant endpoints (18). Furthermore, nitrogen balance and amino acid oxidation provide estimates of total daily amino acid needs but do not address the distribution of protein intake at individual meals. Many of the metabolic roles of amino acids support targeting the quantity of dietary amino acids or protein needs at individual meals distributed throughout the day as opposed to net daily recommendations or an overall percentage of daily energy intake (19).

An alternate approach to nitrogen balance is the indicator amino acid oxidation (IAAO) method (20). The IAAO method is based on the concept that when one indispensible amino acid is deficient, all other amino acids (including a tracer-labeled indicator amino acid) will be oxidized. As the intake of the deficient or limiting amino acid increases, the rate of oxidation of the other amino acids will decline as more amino acids are incorporated into protein. The IAAO method also uses a biphasic regression to calculate a breakpoint for a mean EAR (16). Presumably the IAAO method reflects the minimum amount of protein necessary to create saturation of the transfer RNA (tRNA) for protein synthesis. The point of the lowest oxidation of the indicator amino acid is defined as the requirement for the limiting amino acid. The IAAO method provides estimates of protein requirements at $\sim$1.2 g $\cdot$ kg^{-1} $\cdot$ d^{-1} that are 40–50% higher than nitrogen balance and the current RDA (20).

Although the IAAO method addresses many of the limitations of nitrogen balance, it still targets amino acid oxidation as an undesired metabolic outcome. This is a concept consistent with the efficient use of protein for growth, but there is no evidence that efficiency equates to optimum metabolic health for adults. There are numerous examples of desirable metabolic outcomes as a result of amino acids being consumed in amounts greater than the requirements predicted from the nitrogen balance or IAAO methods. Examples include leucine activation of mammalian/mechanistic target of rapamycin complex 1 (mTORC1) for stimulation of muscle protein synthesis; tryptophan stimulation of serotonin production, which affects satiety or mood; and arginine stimulation of nitric oxide synthase to regulate vascular function. In each case, increases in plasma or intracellular amino acid concentrations trigger the metabolic pathway and amino acid oxidation (21–23). Simultaneous activation of the metabolic signal and the degradation pathway may reflect a feedback loop to protect the signal from chronic exposure and reset the signal for the next meal. Note also that the oxidative catabolism of several EAAs serves to supply important anaplerotic carbon to maintain tricarboxylic acid cycle function (24). Thus, dietary guidelines based solely on the efficiency of amino acid use for growth or nitrogen balance outcomes may be neglecting the fact that changes in intracellular amino acid concentrations and oxidative catabolism are an important means to regulate and support many normal metabolic responses beyond the synthesis of nitrogen-containing compounds.

AMINO ACID ROLES BEYOND RDA DEFINITIONS: LEUCINE AND mTORC1

There are numerous examples of cellular sensing of amino acids and metabolic responses. Mechanisms for cellular sensing of amino acid concentrations occur through tRNA (25), general control nonrepressed 2 (GCN2) (26) and mTORC1 (27). The effect of the branched-chain amino acid (BCAA) leucine on mTORC1 is one of the most investigated signaling responses and highlights the importance of amino acid signaling in normal physiology.

Metabolic pathways for the BCAAs leucine, valine, and isoleucine have been studied extensively since the 1970s when researchers discovered that leucine had a unique role among

EAAs in stimulating muscle protein synthesis (28, 29) and that the liver had minimal capacity to degrade the BCAAs (30). These 2 discoveries highlighted leucine in the relation between dietary protein and optimal muscle mass and function.

BCAAs are relatively small amino acids with aliphatic side chains that are hydrophobic and allow them to exist in tightly coiled positions within proteins. These characteristics allow BCAAs to serve as predominant amino acids in structural proteins such as myosin, fibrinogen, and keratin; in transcription factors known as leucine-zipper proteins; and in globular proteins that require both water-soluble and hydrophobic characteristics such as hemoglobin and myoglobin. In total, BCAAs account for >20% of the amino acids in all proteins, and leucine alone accounts for >8% of all amino acids. Combined with their unique chemical and structural characteristics, the absence of liver capacity to degrade BCAAs ensures that every protein-containing meal produces BCAA-enriched plasma for peripheral tissues (31).

In the 1990s, the development of new methods and reagents for studying the regulation of mRNA translation led to the discovery that leucine activated a signal transduction pathway now known as mTORC1. The discovery of the ability of the BCAAs or leucine alone to stimulate muscle protein synthesis led to a decade of intense research elucidating molecular controls of translation initiation and the associated signal transduction cascades. This research showed that mTORC1 serves to integrate signals from insulin/insulin-like growth factor I (IGF-I) and amino acids to activate key enzymes promoting ribosome biogenesis as well as greater translation efficiency during protein feeding. Numerous reviews are available that describe the mTORC1 signal transduction cascade and activation by dietary leucine (27, 32, 33).

The discovery of the impact of leucine on mTORC1 activation and muscle protein synthesis has been applied to evaluation of dietary protein intake in diverse research settings (21, 24, 34–37). These investigations provided new information leading to the following concepts: *1*) the amount of dietary protein at a meal required to initiate an anabolic response in skeletal muscle is driven by the leucine content; *2*) there is little anabolic benefit in skeletal muscle with protein meals larger than the minimum amount required for maximum mTORC1 signaling and activation of mRNA translation initiation; *3*) the intracellular leucine concentration necessary to trigger muscle protein synthesis also increases BCAA oxidation; and *4*) aging produces "anabolic resistance" requiring increased EAAs to achieve maximum mTORC1 signaling and muscle protein synthesis. These points lead us to propose that the distribution of protein at individual meals is physiologically important to optimize mTORC1 signaling and muscle protein synthesis. This concept is termed the "meal threshold."

The concept of a meal threshold for adult protein is supported by animal and human studies investigating mechanisms regulating muscle protein synthesis. Animal studies that used free leucine or proteins with differences in leucine content showed direct relations of activation of mTORC1 and muscle protein synthesis with dietary leucine (38, 39). These responses require a 2- to 3-fold increase in plasma or intracellular leucine concentrations for maximum activation of mTORC1 and stimulation of muscle protein synthesis (35, 39). Furthermore, once the threshold concentration is achieved, additional leucine has no additional effect on mTORC1 or translation initiation. Human studies confirmed that mTORC1 is a critical regulatory signal for initiating muscle protein synthesis after a meal (40, 41).

A precise leucine threshold for activation of mTORC1 and stimulation of muscle protein synthesis has not been established in human dose-response trials. Animal studies that used large numbers of animals, controlled feeding conditions, and short-term flooding-dose isotope methods showed a leucine threshold for translation initiation (34, 38). However, subject numbers and the use of prolonged steady state isotope methods for determining short-term meal effects limit similar studies in humans. Although dose-response trials are not available, there is a general pattern that appears from clinical trials that meals containing >2.2 g leucine in the form of EAA mixtures (39, 42, 43) or whey protein (43–45) stimulate muscle protein synthesis and meals containing <1.8 g leucine produce little to no response (39, 42–45). It is important to note that these studies were performed in older, sedentary adults and represent the minimum response threshold.

Although activation of mTORC1 is essential for initiation of muscle protein synthesis, mTORC1 does not appear to be a predictor of the duration of muscle protein synthesis after a meal (34, 46, 47). Studies have shown that the mTORC1 signal is activated within 30 min after a meal, with maximum protein synthesis at ~60–90 min. The rate of protein synthesis declines between 2–3 h postmeal, although mTORC1 signals remain activated and plasma leucine concentrations remain elevated (34, 47). The duration of the anabolic period cannot be extended even with continuous infusion of additional EAAs (48). This discordance between the anabolic signals and protein synthesis has been termed a "refractory period," and muscle is thought to require time to recover or reset before a subsequent meal (49).

Protein-containing meals that stimulate mTORC1 also activate the BCAA catabolic pathway (21). This parallel activation of the anabolic pathway of muscle protein synthesis and BCAA oxidation challenges the concept of efficiency of amino acid use as defined by achieving perfect nitrogen balance. Contrary to the RDA goal of minimum nitrogen loss, the maximum anabolic response in skeletal muscle is achieved alongside greater amino acid oxidation (44). This apparent "inefficient" use of amino acids may instead serve as part of the metabolic feedback regulation required to reset the molecular machinery for the next meal. Cellular mechanisms are known to inhibit translation elongation in response to ATP depletion in the muscle (50). The need to allow muscle to recover before the next meal is consistent with the parallel activation of mTORC1 and BCAA catabolism [i.e., activation of the branched-chain keto acid dehydrogenase complex (BCKDC)] (21). Skeletal muscle responds to a leucine-rich meal by initiating the energy-expensive process of muscle protein synthesis, and simultaneously leucine stimulates the BCKDC system to return leucine to premeal baseline levels. Consistent with the increased BCAA oxidation, the leucine degradation pathway serves to stimulate mitochondrial activity to provide energy within the muscle (50).

Sarcopenia is the age-related decline in muscle mass and function and appears to arise, in part, from a reduced capacity for muscle to initiate protein synthesis after a meal (19). The anabolic effect of protein and the threshold at which protein triggers signaling pathways for muscle protein synthesis differ with age, with older individuals requiring a higher intake than younger

individuals. The reduced muscle protein synthesis response to a meal has been termed "anabolic resistance" because of reduced metabolic response to anabolic factors including amino acids, insulin, and resistance exercise (46, 49). Studies have shown that the anabolic resistance can be overcome with meals containing higher amounts of EAAs, and the response appears to be related to the leucine content of the meal. These findings led to dietary recommendations for older adults that emphasize a meal threshold of >20 g protein containing >2.2 g leucine to optimize that anabolic response in skeletal muscle (51).

AMINO ACID SIGNALING AND SATIETY

Other examples of cell sensing of amino acids relate to dietary protein intake, appetite, and satiety. Research evidence has long supported a role for dietary protein in the regulation of food intake. Animals and humans modify food intake associated with diets that have an amino acid imbalance (26), are deficient in an EAA (52), have a low protein density (53), or are rich in protein (54). There is general consensus that meals higher in protein produce greater satiety than meals high in either carbohydrates or fats. These findings translate into reduced food intake at next meals (55) and reduced snacking behavior (56). Proposed mechanisms include mechanical distention of the stomach, incretin responses from the small intestine, neurotransmitter responses in the brain, fuel changes in the hypothalamus, and leptin production (54, 57, 58). Amino acid sensing is believed to contribute to the homeostatic regulation of food intake and body weight.

A concept that encompasses many of these responses is the protein leverage hypothesis (59). The hypothesis states that humans and animals eat to obtain a physiologically desirable amount of protein. When the protein density of the diet is low because of dilution with carbohydrates or fats, both animals and humans consume more total calories to obtain the desired protein. Animal research suggests that signaling pathways exist that link hypothalamic nutrient sensing to behavioral determinants of energy balance (54, 60). A basic mechanism exists to recognize a deficiency of EAAs in the diet and to initiate foraging for dietary sources of these EAAs. The protein leverage hypothesis proposes that humans prioritize protein when regulating food intake and that a decline in the ratio of protein to carbohydrate or fat in the diet drives excess energy intake and could promote obesity (53, 59). A hyperphagic response in rodents given EAA-restricted diets suggests the engagement of such a nutrient-sensing mechanism (52).

The inverse is also true that when the diet is rich in protein, the desired protein amount is obtained with less total food and satiety is high. Evidence to support the protein leverage hypothesis in humans was found in a 12-d randomized crossover study in men and women to determine energy intake, body weight change, and appetite profile in response to changes in the protein to carbohydrate or fat ratios in which individuals reduce total energy consumption when consuming high-protein (30% of energy) diets (61). A longitudinal analysis of a female population found that calories from dietary protein remained more constant over time than calories from dietary carbohydrates or fat, which is consistent with the protein leverage hypothesis (62). The control of food intake is a complex integration of more than one mechanism, and the evidence supports a central role of dietary protein in the regulation of ingestive behavior.

PHYSICAL ACTIVITY ENHANCES THE MEAL RESPONSE TO AMINO ACIDS

Physical activity changes metabolic regulation and amino acid utilization in skeletal muscle (44, 63–66). A single bout of resistance exercise produces increases in both protein synthesis and protein breakdown, but the rate of muscle protein breakdown exceeds synthesis under fasting conditions, resulting in net breakdown of muscle protein and increased amino acid oxidation (64, 65). The ultimate impact of exercise on muscle mass is dependent on protein intake, which can dramatically enhance the rate of muscle protein synthesis in relation to breakdown, resulting in a positive net balance (44, 62).

The rapid changes in muscle protein synthesis after a single bout of exercise suggest regulation through mRNA translation mechanisms. Many of the exercise-induced effects on muscle protein turnover are mediated through insulin and the mTORC1 signaling pathway (63, 66). Numerous studies showed that exercise improves insulin sensitivity in muscle, especially in insulin-resistant conditions of metabolic syndrome or type 2 diabetes (67, 68), and activates mTORC1 signaling and downstream targets including the 70-kDa S6 protein kinase (p70S6K) and the 4E binding protein 1 (4EBP1) (66, 69, 70). Physical activity also modifies the relation of dietary protein to muscle protein synthesis by lowering the threshold at which protein triggers the mTORC1 signaling pathway (44, 45).

The degree to which muscle responds to exercise and dietary protein depends on the type of exercise, timing of the protein meal, quantity of protein, and amount of training. In general, all physical activity has positive effects on maintaining muscle mass and function, but anabolic effects are proportional to resistance and intensity. Exercise at >70% of maximum effort is most often used to induce gains in muscle mass, whereas intensities <20% of maximum effort fail to produce measureable changes in muscle protein synthesis unless the exercise is performed to fatigue (63).

Because exercise appears to enhance anabolic signaling through stimulation of the mTORC1 initiation signal (69), it is logical that protein consumed after exercise would be most beneficial. Although some studies showed benefits of consuming protein before (71) or during (72) exercise, the consensus is that the greatest benefits are observed when protein is consumed after exercise (68, 73). The optimal postexercise timing differs with the degree of training. In untrained subjects, the exercise effects last at least 24 h postexercise, with studies showing that untrained subjects had a greater response to a meal 24 h after a single bout of resistance exercise than did a control group who consumed the same meal but without exercise (41). Furthermore, the exercise response is most dramatic in untrained subjects who produce the largest changes in muscle protein synthesis. The higher the degree of training, the more rapidly the postexercise anabolic response returns to baseline; this implies that a protein meal is most effective when delivered more closely to the exercise, with some studies showing attenuation of training-induced increases in lean mass if protein feeding is delayed beyond 2 h postexercise (74).

The meal threshold for protein to induce muscle protein synthesis is influenced by physical activity and age of the subjects. Acknowledging inherent risks for comparing dietary treatments across studies, the optimum meal threshold for older sedentary adults appears to be >25 g protein (43–45), whereas

healthy, young, active men respond to meals with 15 g protein (63). Furthermore, exercise enhances the protein synthesis response in older adults (44, 45) and appears to reduce the minimum meal threshold (45). Likewise, if protein intakes increase, older individuals can achieve rates of muscle protein synthesis similar to young adults (42–44). Increasing the EAA content of the meal can overcome the anabolic resistance (39), and the effects of exercise and dietary protein appear to be additive for muscle protein synthesis response (45).

Although physical activity enhances the cell's sensitivity to the presence of amino acids, inactivity blunts the activation of the mTORC1 pathway. Short-term bed rest, whether due to hospitalization, illness, or injury, results in a significant loss of lean tissue in both young and older adults (75, 76). Bed rest reduces the meal responses of mTORC1 signaling and amino acid transporter capacity (77). Likewise, declining daily activity also blunts the signaling response. A recent study found that older adults who reduced their daily step-count by ~76% during a 140-d period reduced muscle protein synthesis and increased insulin resistance and the inflammatory markers TNF-α and C-reactive protein (78), but a single bout of resistance exercise before ingesting a sufficient amount of amino acids reverses the anabolic resistance seen during aging (79).

Possible mechanisms for the interaction of exercise with dietary protein include improvements in endothelial function or muscle perfusion, increased amino acid uptake, enhanced insulin sensitivity or amino acid sensing within the cell, and prolonged activation of mTORC1 after exercise (49, 77, 80). High-intensity exercise increases muscle sensitivity to insulin and IGF-I, resulting in prolonged activation of the protein kinase B (PKB)-mTORC1-p70S6K signaling axis that increases the capacity for total muscle protein synthesis (49). Exercise also improves blood flow to muscles and increases receptor sensitivity and amino acid transporters, thereby enhancing delivery of nutrients and hormone signals. Thus, it is clear that exercise, nonexercise physical activity, and age interact to affect tissue sensitivity to amino acid signaling and ultimately dietary protein needs. Dietary recommendations need to more fully consider these variables, which can differ significantly across ages and lifestyles.

SAFE UPPER LIMITS FOR PROTEIN AND AMINO ACID INTAKES

As already indicated, there is a case to be made that the current RDA for protein, which is based largely on minimum amounts needed to avoid deficiency and to maintain growth and development, may not be adequate to support overall metabolic health. With respect to possible negative health effects of protein intakes above the RDA, an Upper Limit (UL) for protein intake, after which intakes become "excessive," should be considered. However, amino acid and protein ULs have not been established and definitions of what constitutes the UL are controversial. Proposed estimates of protein or amino acid ULs include intakes that are just adequate to achieve nitrogen balance, result in a maximal rate of urea synthesis/maximal rate of urea excretion, or yield a maximal rate of oxidation to carbon dioxide (81, 82).

ULs for most amino acids have not been extensively studied, but individual EAAs appear to have high safety limits. Preliminary data exist for leucine, tryptophan, lysine, and methionine. The UL for leucine is reported to be >500 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} or ~38 g/d for a 75-kg individual (83). Leucine, on average, accounts for ~8% of amino acids in protein, which means the UL for leucine equates to a daily intake of ~475 g total protein. Currently, the intake of leucine in the United States is ~8 g/d. The UL for tryptophan has been estimated at ~100–200 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} or ~8–15 g/d (84). Tryptophan accounts for ~1–1.7% of amino acids in protein. A tryptophan intake of 8 g/d equates to >470 g total protein/d, whereas current US intake of tryptophan is ~1 g/d. The UL for lysine is suggested to be 300–400 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} or ~22–30 g/d (85). Lysine accounts for ~6.5% of amino acids in protein, and an intake of 22 g/d equates to ~340/g total protein. The present intake of lysine is ~8 g/d. The UL for methionine is estimated to be ~100 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} or ~7 g/d (86). Methionine accounts for ~2% of amino acids in protein, meaning that an intake of 7 g/d equates to ~350 g total protein/d. The US intake of methionine is ~1.4 g/d. Thus, ULs for amino acids are generally 3- to 5-fold greater than typical intakes in the United States (87), and it is unlikely (without supplementation) that American diets ever exceed the suggested UL for amino acids. Notably, the Institute of Medicine recommends an Acceptable Macronutrient Distribution Range for protein as 10–35% of energy intake, with current US intakes at ~16% of daily energy. Consuming protein in a distributed pattern as described above fits within the Acceptable Macronutrient Distribution Range and the IAAO calculations.

Whereas amino acids appear to have high safety margins as judged by measures of nitrogen metabolism, maximal oxidation, or urea production, the impact of increasing intakes on other metabolic pathways and physiologic systems not measured by these methods should be considered in future research. For instance, higher plasma concentrations of BCAAs are commonly associated with insulin resistance, leading some investigators to propose that excess BCAAs promote metabolic dysregulation (88). Both leucine and insulin stimulate mTORC1, resulting in downstream activation of the p70S6K that is thought to phosphorylate insulin receptor substrate 1 (IRS-1) and reduce insulin sensitivity. Contrary to this perspective, most studies showed that leucine supplementation or leucine-rich diets improve insulin sensitivity (89, 90), and multiple studies for weight loss or diabetes management showed that increasing dietary protein as a substitute for carbohydrates improves insulin sensitivity (6, 91–95). In addition, some studies showed that leucine promotes mitochondrial biogenesis and increased fatty acid oxidation (94, 95). Thus, the elevated blood BCAA profile is quite possibly a biomarker of metabolic dysregulation rather than an initiating event of insulin resistance (89, 90, 96, 97).

Arguably the most frequent concern expressed about higher protein intakes is impairment of renal function. Protein intake beyond the minimum necessary for nitrogen balance promotes urea formation and increases glomerular filtration rate and renal nitrogen load (98, 99). However, there is no evidence that increased urea formation or changes in glomerular filtration rate elicit pathologic outcomes in healthy persons, because clearance becomes more efficient with higher protein intakes (51). For patients with existing kidney disease, the International Society of Renal Nutrition and Metabolism (100) consensus statement recommends that patients consume 0.6–0.8 g/kg body weight if not undergoing dialysis but to increase to 1.0 g/kg during any illness that is catabolic or limits physical activity. For those undergoing dialysis, International Society of Renal Nutrition

and Metabolism recommends daily protein >1.2 g/kg, with at least 50% being of high biological value (100). Higher protein intakes are typically not recommended for individuals with type 2 diabetes because of potentially compromised renal function derived from glucose-induced vascular damage. However, a recent study in overweight and obese individuals with type 2 diabetes who consumed moderate amounts of protein (90–120 g/d) found no negative effects on renal function during a 2-y period (101).

Another concern about long-term use of higher protein diets has been bone health. Increased dietary protein can result in increased urinary calcium, which has been suggested to contribute to bone loss and potential development of osteopenia and osteoporosis. However, the role that protein plays in bone health is far more complex. Studies of the association between protein intake and bone status reported beneficial associations (102), no association (103), and detrimental effects (104), sometimes within the same study population (105). A recent review emphasized a positive effect of protein intake on bone health under conditions of adequate calcium intake (106), whereas a recent clinical trial concluded that there was no effect of high-protein diets on calcium homeostasis during weight maintenance or energy restriction (107). In support of this, a systematic review concluded that the evidence was inconclusive for a significant relation (either positive or negative) for protein intake and bone health (108). It should be noted that calcium and protein interact and both must be adequate to support optimal bone health (109). Substantial evidence exists to establish an association between dietary protein and increased peak bone mass in both young and older adults (102, 105, 106, 109, 110).

In summary, current US intakes are well below proposed ULs that are based on nitrogen balance, maximal amino acid oxidation, or urea production. Additional research is warranted to move beyond these measures to leverage complementary technologies such as metabolomics to form a comprehensive understanding of how increasing intakes of protein above the RDA affects whole-body physiology and metabolism.

MOVING BEYOND THE MINIMUM: OPTIMUM PROTEIN INTAKES FOR ADULT HEALTH

Each metabolic pathway for amino acids has different minimum concentration thresholds for stimulation and maximum capacity. Some amino acid roles such as charging of tRNA for protein synthesis appear to be saturated at cellular concentrations below normal fasted values. The body maintains a high priority for baseline levels of protein synthesis, especially in critical organs such as liver, heart, and diaphragm, and maintains tRNA in fully charged states by using amino acids released from the breakdown of existing proteins. Even during short-term catabolic periods, the liver maintains essential protein synthesis (64).

Other pathways such as the mTORC1 signaling pathway for muscle protein synthesis are dependent on the dietary supply of protein and especially the protein content of meals (34, 41, 46, 63, 111). In studies that examined meal distribution, investigators showed that providing daily protein in one or more large "bolus" or "pulse" meals (>30 g) had positive effects on lean mass or muscle protein synthesis compared with providing the same total amount of protein in a "spread" distribution with multiple small meals (<20 g) or continuous intragastric infusion (17, 112–116).

Most adults in the United States have an unbalanced meal distribution of protein (18) with >60% of daily protein consumed during a single evening meal and ≤15 g at breakfast (117, 118). If the protein distribution (and quantity of leucine) is critical for optimum muscle protein synthesis, the typical American meal pattern should be altered to maximize metabolic health. The average protein intake for men >20 y is ~98 g/d and for women is ~68 g/d, but the unbalanced distribution may reduce the effectiveness of the daily intake for muscle health and modulate ingestive behavior. Also not addressed in existing protein recommendations are the changes that occur in amino acid utilization as a result of aging, with increased physical activity, or with physical inactivity.

This article focused on metabolic regulation and outcomes that suggest that protein should be distributed in specific amounts at meals. The current hypothesis is that the regulation of protein synthesis determine net balance of protein turnover except during extreme catabolic conditions (63). To our knowledge, there are no data concerning protein breakdown and the impact of dietary protein distribution at meals (119), but this is an important area for future research.

In summary, there is a need to review how protein requirements should be defined or expressed. The difference between minimum compared with optimum protein intakes and the concept of a meal-based protein threshold for adults are not addressed in current dietary recommendations. In the 2010 *Dietary Guidelines for Americans* (11), protein needs are expressed as a percentage of energy intake (% kcal), and the 2002 Dietary Reference Intakes define the RDA as grams per kilogram of body weight per day ($g \cdot kg^{-1} \cdot d^{-1}$). However, new research emphasizes the relevance of protein grams per meal, and a case can be made that a greater focus on EAA intakes is warranted to achieve optimal health outcomes. The consensus position paper from the PROT-AGE Study Group recommended daily protein intake of >1.0 g/kg but emphasized the need to focus on meal quantity and timing of protein as important factors in adult health (51). Additional research is needed to define specific total daily and meal-based protein intakes that affect health indexes and to better refine these indexes by considering nontraditional outcome variables such as muscle and lean body mass function, mitochondrial dynamics, and tissue fuel partitioning.

The authors' responsibilities were as follows—All of the authors participated in Protein Summit 2.0 and were involved in the writing and editing of the manuscript; and DKL: wrote the first draft of the manuscript and had primary responsibility for the final content. None of the authors had a conflict of interest.

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