Dietary Protein Impact on Glycemic Control during Weight Loss

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ABSTRACT Diets with higher protein (1.5 g · kg⁻¹ · d⁻¹) and reduced carbohydrates (120 to 200 g/d) appear to enhance weight loss due to a higher loss of body fat and reduced loss of lean body mass. While studies of prolonged use of moderate protein diets are not available, short-term studies report beneficial effects associated with increased satiety, increased thermogenesis, sparing of muscle protein loss, and enhanced glycemic control. Combined impacts of a moderate protein diet are likely derived from lower carbohydrates resulting in lower postprandial increase in blood glucose and lower insulin response, and higher protein providing increased BCAA leucine levels and gluconeogenic substrates. A key element in the diet appears to be the higher intake of BCAA leucine with unique regulatory actions on muscle protein synthesis, modulation of the insulin signal, and sparing of glucose use by stimulation of the glucose-alanine cycle. This review focuses on the contributions of leucine and the BCAA to regulation of muscle protein synthesis and glycemic control. J. Nutr. 134: 968S–973S, 2004.

KEY WORDS: • obesity • insulin • leucine • BCAA

There is general consensus that the most critical factor in weight management is total energy intake. Yet the ideal balance of macronutrients for weight loss and adult weight management remains widely disputed. Often this debate focuses on the relative merits or risks of carbohydrates vs. lipids. However, when the energy content of the diet is equal, the relative levels of carbohydrates and lipids in the diet appear to have minimal affect on either weight loss or body composition (1–3). On the other hand, there is increasing evidence that diets with reduced levels of carbohydrates and higher levels of protein may be beneficial for weight loss (4–10). These studies report that diets with reduced carbohydrates and higher protein appear to increase weight loss (4–6,8,9), increase loss of body fat (5,6,8), or reduce loss of lean body mass (4,6,8,10). While potential benefits for higher protein diets during weight loss are emerging, a metabolic explanation for optimal levels of carbohydrates and proteins remains unknown (11).

Possible explanations for the beneficial effects of diets with higher protein and reduced levels of carbohydrates include lower energy intake associated with increased satiety (5,7,9,12), reduced energy efficiency or increased thermogenesis (6,13), sparing of muscle protein loss (8,14), and enhanced glycemic control (8,10). Our research has focused on the role of amino acids in regulation of muscle protein metabolism (8,15,16) and glycemic control (17,18). This presentation is limited to these topics.

The role of protein in the diet is to provide the 20 naturally occurring amino acids and specifically to provide the 9 indispensable amino acids. Each of these amino acids has a unique requirement as a building block for body proteins. However, the dietary requirement is not tightly linked to substrate needs for protein synthesis. One reason for the lack of a direct relationship is the recycling of amino acids after degradation of existing proteins. Amino acids are efficiently reutilized for synthesis of new proteins. Even during maximum rates of growth the body deposits ~10 g of protein per d (19). Hence the dietary protein needed to maintain essential protein turnover appears quantitatively small and with no clear metabolic relationship to the current RDAs (recommended dietary allowance) of 0.8 g · kg⁻¹ · d⁻¹.

Beyond the needs for amino acids required for synthesis of new proteins, amino acids participate in numerous metabolic roles. In many cases the significance of these pathways is proportional to dietary intake, such as dietary intake of tryptophan or phenylalanine (i.e., tyrosine) as precursors to neurotransmitters with dietary intake potentially impacting appetite regulation (20,21), or intake of arginine altering epithelial production of nitrous oxide and cell signaling pathways (22,23). Another example of an amino acid with metabolic roles proportional to dietary intake is the BCAA leucine with potential regulatory roles on skeletal muscle protein synthesis and glycemic control (16,17).

Leucine exhibits an array of metabolic roles. Like all amino acids, leucine is essential for protein synthesis. Based on nitrogen-balance measurements, the requirement for leucine to...
maintain short-term stability of body protein is \( \sim 1 \) to 3 g per d (11,24). However, leucine participates in numerous other metabolic processes including serving as a fuel for skeletal muscle (25,26), modulation of the intracellular insulin/phosphatidylinositol-3 kinase (PI3-K)\(^3\) signaling cascade (27,28), a unique regulator of muscle protein synthesis (29,30), and serving as a donor of an amino group for production of alanine or glutamine (31,32). In each of these pathways, the impact of leucine is proportional to availability and is dependent on its intracellular concentration. To optimize these pathways, we estimate that the leucine requirement is \( >8 \) g/d (8,33,34). Leucine is relatively abundant in the food supply, accounting for \( \sim 8\% \) of dietary protein with dairy products being particularly rich in leucine and the BCAA (Table 1). This range of leucine intake is reasonable within the guidelines of the dietary reference intakes (11). These metabolic roles for leucine form the bases for our hypothesis for the importance of increased dietary protein during weight loss (8,17).

### Leucine and regulation of muscle protein synthesis

The role of leucine in muscle protein synthesis is different from other essential amino acids. During catabolic periods such as energy restriction, supplementation with leucine or a complete mixture of the 3 BCAAs, leucine, isoleucine, and valine, stimulates muscle protein synthesis (35–37). This research suggests a regulatory role of leucine that is dependent on intracellular concentration and is different from traditional substrate roles for protein synthesis or nitrogen balance (36,38,39). We found that leucine supplementation stimulates recovery of muscle protein synthesis during food restriction or after endurance exercise (38,39).

The molecular mechanisms for the actions of leucine in protein synthesis are now known to involve regulation of phosphorylation events and components of the insulin signaling pathway. The site for leucine action is a kinase in the insulin signaling cascade previously identified as mTOR (mammalian target of rapamycin) (Fig. 1). This regulation was first recognized associated with translational control of muscle protein synthesis (28,38). Increases in leucine concentration stimulate mTOR kinase activity for phosphorylation control of the eIF4 initiation complex and of the S6 ribosomal protein. Specifically, leucine stimulates phosphorylation of the inhibitory binding protein (4E-BP1) causing the binding protein to dissociate from the eIF4E translational initiation factor. After dissociation, eIF4E is available to bind with eIF4G and form the active initiation complex (Fig. 1). Leucine via mTOR also increases activation of p70\(^{56} \)S6 kinase leading to phosphorylation of the S6 ribosomal protein and enhanced global rates of protein synthesis (39). The mechanisms for translational regulations by leucine have been recently reviewed (29,30). This unique role of leucine in regulation of muscle protein synthesis is consistent with the sparing of lean body mass seen with use of higher protein diets during weight loss (8,10,14).

### Regulation of blood glucose

Before examining roles for amino acids in glucose homeostasis, 2 concepts for regulation of blood glucose concentration will be reviewed briefly. In a fasted condition, the liver is the sole source of endogenous glucose production (EGP) and maintains a rate of production (or rate of appearance (Ra)) of \( \sim 5 \) to 7 g of glucose per h. Hepatic glucose production is derived from a combination of glycogen breakdown and gluconeogenesis. The relative contribution of these 2 pathways has been debated; however, gluconeogenesis appears to be the predominant pathway accounting for up to 75% of EGP (40–42). The specific contribution from gluconeogenesis is influenced by dietary factors including the duration of the fasted period and previous dietary intakes of carbohydrates and protein. During nonabsorptive conditions, the rate of hepatic glucose production is exactly balanced with the rate of glucose use by peripheral tissues under basal insulin levels ranging from 5–20 \( \mu U/mL \) (35–140 pmol/L). During periods of food intake and absorption of exogenous glucose, hepatic release of glucose into the blood may exceed 30 g/h (41). The rise in dietary glucose in portal circulation and the increased rate of appearance of glucose from the liver into the blood stimulate release of insulin to accelerate peripheral disposal of glucose in skeletal muscle and adipose tissue. The increase in insulin also serves to reduce EGP by inhibiting hepatic gluconeogenesis and glycogen breakdown. In nondiabetic conditions, the net balance of hepatic release and peripheral uptake limits the rise in postprandial blood glucose to \( <7.77 \) mmol/L.

Critical questions in evaluating the balance of protein and glucose metabolism are the time course of glucose concentration following a meal, the rates at which glucose enters peripheral tissues, and the rate at which glucose is released into the blood. Figures 2 and 3 illustrate these questions and pathways for glucose metabolism (3). At the molecular level, there is substantial evidence that leucine, as a BCAA, plays a role in these glucose metabolism pathways.

### Table 1

Leucine and BCAA content of foods

<table>
<thead>
<tr>
<th>Food Type</th>
<th>Leucine (%)</th>
<th>BCAA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey protein isolate</td>
<td>14%</td>
<td>26%</td>
</tr>
<tr>
<td>Milk protein</td>
<td>10%</td>
<td>21%</td>
</tr>
<tr>
<td>Egg protein</td>
<td>8.5%</td>
<td>20%</td>
</tr>
<tr>
<td>Muscle protein</td>
<td>8%</td>
<td>18%</td>
</tr>
<tr>
<td>Soy protein isolate</td>
<td>8%</td>
<td>18%</td>
</tr>
<tr>
<td>Wheat protein</td>
<td>7%</td>
<td>15%</td>
</tr>
</tbody>
</table>

1 Values reflect g of amino acids/100 g of protein. Source: USDA Food Composition Tables.

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**Abbreviations used:**
- AUC, area under the curve
- BCKAD, branched-chain ketoacid dehydrogenase
- 4E-BP1, inhibitory binding protein
- EGP, endogenous glucose production
- GLUT1, insulin independent glucose transporter
- IRS-1, insulin receptor substrate
- PKC, protein kinase C
- p70S6, mammalian target of rapamycin
- PI3-K, phosphatidylinositol-3 kinase
- Ra, rate of appearance

**FIGURE 1** Insulin signaling cascade. GLUT1, insulin independent glucose transporter; PKC, protein kinase C; eIF, translational initiation factors.
carbohydrate in the diet concern the fundamental assumption about the ideal regulation of blood glucose. If the assumption is that insulin is the primary regulator of blood glucose, then the experimental model is likely to be based on evaluating factors affecting the ability of insulin to handle an exogenous bolus of carbohydrates. However, if the assumption is that the liver is the primary regulator of blood glucose then the ideal model is focused on hepatic control of the rate of glucose appearance and regulation of the rate of gluconeogenesis. The correct model is likely different for a 22 y old athlete with high muscle activity consuming a 3500 kcal/d (14653 kJ/d) diet vs. a sedentary 53 y old attempting to restrict energy intake to 1700 kcal/d (7117 kJ/d) to achieve weight loss. The decision about the correct model underpins the dietary decision about the ideal mixture of protein vs. carbohydrates during weight loss.

Figure 2 illustrates a theoretical oral glucose response curve plus a parallel insulin response curve. These curves represent responses to a meal providing ~400 kcal (1675 kJ). At time zero (fasted), the Ra for glucose is low; fasting blood glucose is 4.7 to 5.0 mmol/L; insulin is at basal levels; and regulation of blood glucose is predominately hepatic. With the beginning of a high carbohydrate meal, there is a rapid rise in blood glucose to a maximum of 7.77 mmol/L and then a return to fasted concentrations after 120 min. The time frame for an OGRC is dependent on the size and composition of the meal. The meal response for insulin follows a similar time course with fasting insulin of ~15 μU/mL (105 pmol/L) increasing rapidly to an early peak of perhaps 60 to 80 μU/mL (430–575 pmol/L) and then a prolonged slow return to fasting levels. These curves highlight 2 important periods of glycemic control, fasted or nonabsorptive periods at times 0 and 120 min, and the absorptive period. During the nonabsorptive periods, hepatic production releases glucose in proportion to peripheral use and blood glucose remains stable. During the absorptive period, there is a high rate of appearance of exogenous glucose. As the glucose appears in the blood, insulin is released to coordinate the rate of glucose disposal with the rate of appearance. Under conditions of insulin resistance as seen in type-2 diabetes this regulation is ineffective and blood glucose continues to rise often well above 11.1 mmol/L.

Assuming that insulin is the primary regulator of blood glucose, then experiments are often designed to evaluate peak insulin response or area under the curve (AUC) of an oral glucose response curve. For this approach, 1 of the most useful techniques has been the hyperinsulinemic, euglycemic clamp (43). This method requires insulin to be infused into a peripheral vein at a constant rate to stabilize blood insulin at 80–150 μU/mL (575–1075 pmol/L). Then glucose is infused at rates that are adjusted to achieve stable concentration of blood glucose at ~7.77 mmol/L. Under these conditions, the rate of infusion of glucose equals the rate of glucose disposal by tissues. For type 2 diabetes, a much lower level of glucose would be infused reflecting a lower rate of peripheral glucose clearance and insulin insensitivity. Using the same insulin model and the euglycemic clamp, it is possible to test the effects of infusion of amino acids on glycemic control. Investigators using this approach demonstrate that amino acid infusion reduces glucose uptake leading to the conclusion that increased amino acid availability produces insulin insensitivity and inhibits glucose utilization (44).

Impact of amino acids on glycemic control

Interactions of amino acids with carbohydrate metabolism have been recognized for years; however, the research literature is unclear whether dietary protein has a positive or negative impact on glycemic control. Amino acids directly contribute to de novo synthesis of glucose via gluconeogenesis and participate in re-cycling of glucose carbon via the glucose-alanine cycle. Amino acids including arginine and leucine stimulate insulin release from the pancreas; leucine also appears to modulate the intracellular insulin signal in skeletal muscle and adipose tissue (45). The net impact of amino acids on glycemic control appears to be dependent on the experimental approach and the amount of amino acids used.

In 1927 Sweeney (46) reported that young adults fed diets high in protein displayed reduced ability to dispose of oral glucose. Specifically, subjects were fed test diets for 2 days that were either mostly carbohydrates (e.g., bread, potatoes, rice and oatmeal) or mostly protein (e.g., lean meats and egg whites). On d 3, subjects were tested for their response to an oral glucose tolerance test using ~100 g of glucose (1.75 g/kg) and blood was obtained at 0, 30, 60, and 120 min. Subjects preconditioned with the carbohydrate diet exhibited peak plasma glucose concentrations of 6.66 mmol/L, while subjects preconditioned with the protein meals had peak glucose concentrations of >8.88 mmol/L. These data suggest that a high protein diet decreases oral glucose tolerance.

Later studies reported that amino acids decrease glucose disposal, induce hyperinsulinemia and hyperglycemia, and potentially lead to insulin resistance (44,47–49). Most of these studies used direct intravenous infusion of amino acids into the human forearm under fasted conditions and used euglycemic clamp techniques to measure glucose uptake and insulin resistance. Using these techniques, investigators found that acute increases in plasma amino acid concentrations resulted in higher plasma glucose concentrations, lower glucose uptake, and elevated plasma insulin levels (44,47,48). Possible mechanisms for these actions include competition between amino acids and glucose as oxidative substrates (47,48,50) or modulation of the insulin response including reduced glucose uptake or direct interaction with early steps in insulin signaling (27). These studies used supraphysiological concentrations of insulin and amino acids. While these acute conditions are useful for discerning increments in insulin sensitivity, the physiological relevance of these supraphysiological concentrations administered i.v. for predicting response to chronic dietary conditions is unclear.

One of the first studies of the differences in amino acid metabolism between i.v. administration and oral intake was by Floyd et al. (51,52). These investigators evaluated the insulin
response to i.v. infusion of amino acids or glucose (51) and also examined the insulin response to oral intake of protein (52). They found that infusion of 30 g of amino acids produced a 3-fold higher insulin response (~180 μU/mL) than infusion of 30 g of glucose (~50 μU/mL), suggesting a dramatic hyperinsulinemic effect of amino acids. However, these investigators also examined the same measurements after subjects consumed a meal of 500 g of beef liver and found that the peak insulin response to the protein meal was only 30 μU/mL. Assuming that leucine is 1 of the most potent insulin secretagogues, the i.v. infusion provided <5 g of leucine while the beef meal provide >14 g of leucine (52). These data suggest that amino acids have minimal impact on plasma insulin concentrations when entering the body via the GI tract.

Nuttall and Gannon (53–55) reported similar findings. Using isoenergetic meals, they demonstrated that substituting dietary protein for carbohydrates reduced the meal responses of both plasma glucose and insulin (53). Likewise, they reported that consumption of a test meal containing 50 g of protein (consumed as lean beef) vs. 50 g of glucose that the protein intake alone had essentially no impact on basal blood glucose concentrations and the insulin response to the meal was <20% of the response with a comparable energy intake from glucose (54). While these results are intuitively obvious, they directly contradict the findings that protein is hyperinsulinemic and hyperglycemic.

Explanations for the differences in handling of i.v. vs. oral amino acids involve diverse metabolic pathways. Unlike glucose, which appears in the blood rapidly after a meal, amino acids are slow to leave the gut (56), extensively modified in composition by the gut and liver (13), and appear in the blood slowly with metabolism over an extended postprandial period (48). Metabolism of dietary amino acids by the gut and liver has a major impact on the amino acid profile reaching systemic circulation. Specific examples include removal of nearly 100% of dietary glutamine and glutamate, 60% of threonine, and 40% of phenylalanine during the absorption process largely by oxidative degradation (13). The primary exceptions to this pattern of modifications are the BCAAs, with over 80% of dietary content of leucine, valine, and isoleucine directly reaching blood circulation. A second important issue in considering amino acid metabolism compared with glucose handling is the time course. For glucose, the postprandial handling occurs mostly within the first 2 h (43); however for amino acids the rate of disposal is much slower with <20% of the dietary amino acids degraded within the first 2 h (48). Thus, direct comparison of a high carbohydrate diet vs. a high protein diet is that the carbohydrate diet requires rapid equilibration of the glucose and insulin metabolic system with dramatic shifts between hepatic vs. peripheral regulations, while the diet serves to stabilize the glycemic environment with delayed metabolism and less reliance on peripheral insulin actions.

**Glycemic control with moderate protein, moderate carbohydrate weight loss diets**

Diets with reduced carbohydrates and higher protein produce lower meal responses for glucose and insulin (10,18,54). During studies of weight loss, we found that adult women maintained on a moderate protein diet for 10 wk had more stable blood glucose after an overnight fast and at 2 h after a test meal (8,18). The moderate protein diet also appeared to stabilize the insulin response to a test meal, while subjects receiving an isoenergetic diet high in carbohydrates increased the insulin needed to respond to a test meal. Similar meal responses were reported by Farnsworth et al. (10). They found that after 16 wk subjects consuming a moderate protein weight loss diet displayed lower meal responses for peak glucose and insulin concentrations and total AUC after test meals. These data suggest that diets with reduced carbohydrates and higher protein stabilize glycemic control during weight loss.

We observed an additional example of this effect with subjects exhibiting elevated postprandial insulin responses (Layman, unpublished data). During preliminary screening of overweight subjects, we identified 10 subjects with abnormally high insulin responses at 2 h after a test meal (Fig. 3). Normal values for a 2-h postprandial response are ~5–15 μU/mL above basal insulin concentrations, while these subjects averaged 76 μU/mL above basal levels. Subjects were paired for body weight and insulin values and randomly assigned to either the moderate protein or high carbohydrate diet. After consuming the respective diets for 4 and 10 wk, we evaluated insulin responses to the test meals. As expected, as the subjects lost weight (~6.3 kg) during the 10-wk energy restriction and they improved glycemic control as measured by reduced postprandial insulin response to the test meal. For the CHO Group, average values at wk 0 = 77 μU/mL and at wk 10 = 38 μU/mL. On the other hand, subjects consuming the moderate protein diet achieved normal values for 2-h insulin response after only 4 wk on the diet with average values at wk 0 = 75 μU/mL and at wk 10 = 12 μU/mL. These changes appear to be beneficial associated with the overall risk patterns of obesity and Metabolic Syndrome (57,58).

Reasons for enhanced glycemic control with use of moderate protein diets remain to be fully elucidated; however, elements of possible regulations have been established. The overall contributions of dietary amino acids to glucose homeostasis were established by quantitative evaluations of hepatic glucose production. Jungas et al. (59) reported that amino acids serve as a primary fuel for the liver and the primary carbon source for hepatic gluconeogenesis. Other investigators found that gluconeogenesis provides >70% of fasting hepatic glucose release, with amino acids serving as the principal carbon source (40,41). Estimates of the contribution of amino acid carbon to de novo glucose synthesis range from 0.6 to 0.7 g of glucose from 1 g of dietary protein (13,55). In addition to the direct conversion of amino acid carbon to gluconeogenesis precur-

![Figure 3](image-url)
sors, there is also the contribution of the BCAA to glucose recycling via the glucose-alanine cycle (31,32). There is a continuous flux of BCAA from visceral tissues through the blood to skeletal muscle where transamination of the BCAA provides the amino group for production of alanine from pyruvate with a corresponding movement of alanine from muscle to liver to support hepatic gluconeogenesis. Although the impact of the glucose-alanine cycle has been debated, Ahlborg et al. (31) reported that alanine accounted for 40% of endogenous glucose production during prolonged exercise. Under normal conditions, alanine arising from BCAA nitrogen likely accounts for about 25% of gluconeogenesis from amino acids (17). These studies provide evidence for the linkage between dietary protein and glucose homeostasis.

A focus on leucine

The interactions between amino acids and glycemic control are influenced by total dietary protein providing substrates for gluconeogenesis and by total intake of BCAA determining the capacity for glucose-alanine cycling. Within these requirements, leucine serves as a selective marker for intracellular recognition of the quantity and/or quality of protein in the diet. Peripheral tissues have a unique ability to sense the intracellular leucine concentration. Increases in leucine trigger an array of phosphorylation events that serve to maintain skeletal muscle mass and limit oxidative use of glucose by muscle. The combination of circulating insulin and tissue levels of leucine allow skeletal muscles to manage protein metabolism and fuel selection in relation to diet composition.

As outlined above, leucine stimulates translational regulation of muscle protein synthesis through modulation of downstream elements of the insulin/PI3-k signal pathway (Fig. 1). Higher leucine stimulates mTOR kinase activity and phosphorylation of the inhibitory binding protein 4E-BP1 and p70S6 kinase. The mechanism allowing mTOR to respond to leucine concentrations likely involves a secondary protein with a potential candidate identified as rapTOR (60). This relationship allows for skeletal muscle to sense the quantity or quality of dietary protein and to adjust the rate of muscle protein synthesis in proportion to the availability of substrate.

Parallel with mTOR actions on translational initiation factors, mTOR has been shown to stimulate upstream phosphorylations of insulin receptor substrate (IRS-1) potentially altering the insulin receptor signal (27,28). These findings have been suggested as possible explanations of the amino acid-induced insulin resistance observed with euglycemic clamps (27,44). We observed similar effects in muscle using oral gavage of leucine. Our preliminary findings suggest that leucine induces mTOR phosphorylation resulting in downstream phosphorylations of 4E-BP1 with activation of the initiation factors and upstream phosphorylations of IRS-1 with decreased activity of the PI3-K complex (61). However, we found no change in the rate of glucose uptake into the muscles. These data suggest that potential downregulation of the insulin signal does not produce negative outcomes on either rates of protein synthesis or glucose transport. Possible explanations for the apparent disconnect of the insulin signal with glucose transport may be that under sedentary conditions basal levels of insulin dependent glucose transporter (GLUT4) on the cell membrane are adequate to maintain glucose transport, or that levels of the noninsulin dependent GLUT1 are important for baseline levels of glucose transport. An additional possibility is that mTOR phosphorylation of IRS-1 is a component of the normal feedback regulation of the insulin signal and decreased activity of PI3-K represents degradation of the signaling complex. In support of this possibility, we observed that the decrease in PI3-K activity was greater at 60 min after oral gavage than at 30 min suggesting an initial activation with accelerated degradation of the IRS1-PI3-K complex (61).

Leucine also serves as a metabolic signal for fuel choices. Increases in the intracellular concentration of leucine appear to be the primary regulator of the branched-chain ketoacid dehydrogenase (BCKAD), the rate-limiting step in oxidation of the BCAA (62). An increase in leucine raises the concentration of its keto-analogue α-ketoisocaproate, a potent inhibitor of the BCKAD kinase that is responsible for inactivation of the BCKAD by phosphorylation. Inhibition of the BCKAD kinase leaves the BCKAD phosphatase unopposed, resulting in dephosphorylation and activation of the BCKAD. The BCKAD stimulates decarboxylation of the 3 BCAAs and commits them to oxidation. At the same time, the rise in leucine concentration also inhibits pyruvate dehydrogenase (50), limiting pyruvate oxidation and moderating glucose degradation by skeletal muscle (48). Thus when intracellular levels of leucine are elevated, muscle has the potential to use glucose derived from either the blood or muscle glycogen as a glycolytic fuel and then trap the pyruvate carbon as alanine via transamination with amino acid-nitrogen derived from BCAA. These mechanisms appear to be particularly important during periods of low energy intake or endurance exercise when BCAAs are increased in muscle, insulin is low, and sparing of blood glucose is important (17,25,26).

In summary, use of diets with higher protein and reduced carbohydrates appears to enhance weight loss with greater loss of body fat and reduced loss of lean body mass. Beneficial effects of high protein diets may be increased satiety, increased thermogenesis, sparing of muscle protein loss, and enhanced glycemic control. Specific mechanisms to explain each of the observed outcomes remain to be fully elucidated. We suggest that a key to understanding the relationship between dietary protein and carbohydrates is the relationship between the intakes of leucine and glucose. Leucine is now known to interact with the insulin-signaling pathway with apparent modulation of the downstream signal for control of protein synthesis resulting in maintenance of muscle protein during periods of restricted energy intake. Leucine also appears to modulate glucose use by skeletal muscle. While total protein is important in providing substrates for gluconeogenesis, leucine appears to regulate oxidative use of glucose by skeletal muscle through stimulation of glucose recycling via the glucose-alanine cycle. These mechanisms appear to provide a stable glucose environment with low insulin responses during energy-restricted periods.

LITERATURE CITED


Potential Importance of Leucine in Treatment of Obesity and the Metabolic Syndrome\textsuperscript{1–3}

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ABSTRACT Diets with total protein intake $>1.5$ g kg$^{-1}$ d$^{-1}$ and carbohydrate intake $<150$ g d$^{-1}$ are effective for treatment of obesity, type 2 diabetes, and the Metabolic Syndrome. These diets improve body composition and enhance glycemic control. During weight loss, protein-rich diets reduce loss of lean tissue and increase loss of body fat. Specific mechanisms to explain each of these clinical outcomes remain to be fully elucidated. We propose that keys to understanding the relationship between dietary protein and carbohydrates are the relationships between the branched-chain amino acid leucine and insulin and glucose metabolism. Leucine is known to interact with the insulin signaling pathway to stimulate downstream signal control of protein synthesis, resulting in maintenance of muscle protein during periods of restricted energy intake. Leucine also appears to modulate insulin signaling and glucose use by skeletal muscle. Whereas total protein is important in providing substrates for gluconeogenesis, leucine appears to regulate oxidative use of glucose by skeletal muscle through stimulation of glucose recycling via the glucose-alanine cycle. These mechanisms produce protein sparing and provide a stable glucose environment with low insulin responses during energy-restricted periods. J. Nutr. 136: 319S–323S, 2006.

KEY WORDS: • diabetes • glycemic control • insulin • protein synthesis

Evidence is accumulating that diets with higher protein and reduced carbohydrates are beneficial for weight loss (1–7). These studies report that diets with reduced ratios of carbohydrates to protein increase weight loss (1–3,5,6), increase loss of body fat (2,3,5), and reduce loss of lean tissue (1,3,5,7). We proposed that the beneficial effects of a higher protein diet included the roles of leucine in sparing muscle protein loss and enhancing glycemic control (8,9). This article will review metabolic roles of leucine and examine some of the clinical trials with higher protein diets and weight loss.

The BCAA leucine plays multiple roles in metabolism beyond the minimum requirement as an essential substrate for synthesis of new proteins (8,10). These roles include a key regulator of translation initiation of protein synthesis in skeletal muscle (11), a modulator of insulin/PI3-kinase signaling (12,13), a fuel for skeletal muscle (14), and a primary nitrogen donor for production of alanine and glutamine in skeletal muscle (15). The potential for leucine to impact protein synthesis, insulin signaling, and production of alanine and glutamine is dependent on dietary intake and increasing leucine concentration in skeletal muscle (8,10,13).

The multiple roles of leucine are, at least in part, associated with absence of the branched-chain aminotransferase enzyme in liver, resulting in an enriched supply of the BCAA appearing in blood (8,10,16). Dietary BCAs reach the blood virtually unaltered from levels in the diet, allowing leucine to reach skeletal muscle in direct proportion to dietary intakes. This is a striking metabolic difference for these amino acids, which account for $>20\%$ of total dietary protein. Using the traditional thinking that dietary protein requirements should be defined by efficiency of nitrogen handling, we are left to ponder why the body evolved to metabolize $20\%$ of total amino acids (and total nitrogen) in peripheral tissues? We hypothesized that this unique treatment of the BCAA and specifically leucine provides an important signal of dietary quality for skeletal muscle (8,10).

One of the first reports that leucine metabolism was optimized at dietary intakes greater than the minimum recommended dietary allowance (RDA) was provided by the MIT group (17). Using stable isotopes and measurement of whole-body leucine metabolism, daily leucine usage was determined to be $>6$ g d$^{-1}$ and $\sim 3$ times higher than recommended dietary...
allowance values based on nitrogen balance. This difference highlights the important conceptual difference between requirements based on minimum levels to prevent deficiencies versus optimum levels for metabolic balance. These findings were confirmed by Pencharz and Ball (18,19), who determined rates of leucine oxidation and estimated daily leucine needs at ~8 g/d.

**Leucine regulation of muscle protein synthesis**

Unique metabolic roles for leucine were reported first for regulation of skeletal muscle protein synthesis (20). During catabolic periods, such as fasting or energy restriction, supplementation with leucine or a complete mixture of the three BCAAs leucine, isoleucine, and valine stimulates muscle protein synthesis (8,11). Likewise, leucine supplementation stimulates recovery of muscle protein synthesis after exercise (21,22). The molecular mechanisms for the actions of leucine in protein synthesis are now known to involve regulation of phosphorylation events and components of the insulin signaling pathway in translational control of muscle protein synthesis (11,23).

One site for leucine action is a kinase in the insulin signaling cascade previously identified as mammalian target of rapamycin (mTOR) (8,11,12,23). Increases in leucine concentration stimulate mTOR kinase activity for phosphorylation control of the eukaryotic initiation factor 4 complex and the S6 ribosomal protein (Fig. 1). Leucine stimulates phosphorylation of the inhibitory binding protein 4E-BP1, causing the binding protein to dissociate from the eukaryotic initiation factor-4E (eIF4E) translational initiation factor (11). Leucine stimulates activation of a second initiation factor eukaryotic initiation factor-4G (eIF4G) through an mTOR-independent kinase (11). Phosphorylated eIF4G is available to bind with free eIF4E to form the active initiation complex. Furthermore, leucine via mTOR activates p70S6 kinase, leading to phosphorylation of the S6 ribosomal protein (Fig. 1) (11,23). Mechanisms for translational regulations by leucine have been reviewed previously (11).

Molecular mechanisms for leucine stimulation of protein synthesis are supported by human studies (24–26). Short-term (2 to 4 h) intravenous infusions of large doses (3 to 7 g) of leucine produce anabolic changes in protein turnover and nitrogen balance (25,26). Likewise, leucine stimulates protein synthesis during catabolic conditions produced by short-term food deprivation or exhaustive exercise (24,27). After an overnight fast or intense exercise, protein synthesis is reduced compared with the rate of protein breakdown, producing a net breakdown of muscle protein. This catabolic period continues until adequate protein or specifically leucine is consumed to increase plasma and intracellular leucine concentrations (28). Oral intake of 2.5 g of leucine stimulates muscle protein synthesis after exercise or an overnight fast (24,27). These studies support the role of leucine as a key amino acid for reversing catabolic conditions.

Although leucine stimulates muscle protein synthesis in acute studies, the efficacy of prolonged administration is less clear. Human clinical trials with liver disease or sepsis have not produced consistent long-term benefits with leucine administration (25,26). Although these studies are complicated by the disease states, a sustained anabolic effect of leucine should be noted when plasma and intracellular concentrations of leucine are reduced, such as during fasting or energy restriction for weight loss. To test this hypothesis, one approach would be to use a low protein diet control and test the effects of supplemental leucine. This approach would mimic protocols of acute studies. Unfortunately, prolonged feeding of a low protein diet supplemented with leucine increases catabolism of valine and isoleucine, producing an amino acid imbalance among the BCAAs (10).

A second experimental approach would be to feed a low protein diet and provide a balanced supplement of the three BCAAs. This approach stimulates short-term increases in protein synthesis but depletes plasma concentrations of other essential amino acids (29,30). Because of the limitations of these approaches, we elected to test the long-term potential of leucine to reduce muscle wasting during weight loss using diets with mixtures of high-quality proteins that provided >8 g/d of leucine (5).

**Protein-rich diets for treatment of obesity**

An initial concept of protein sparing during weight loss was demonstrated in a short-term study by Bistrian et al. (31). They utilized very low energy diets providing 1.5 g · kg\(^{-1}\) · d\(^{-1}\) of protein to produce rapid weight loss and found high protein diets reduced urinary nitrogen losses. Conclusions were reached that high protein intake was beneficial in minimizing wasting of lean tissue during weight loss for obesity.

In another short-term study, leucine infusion was shown to reduce nitrogen loss in obese subjects fasting to obtain weight loss (32). Over a 4-h period, ~3.5 g of leucine was infused, resulting in reduction of 24-h nitrogen losses. These studies suggest a higher protein diet can be beneficial during weight loss and provide evidence that protein sparing effects are largely derived from leucine.

Using these findings, we conducted two weight loss trials using diets designed to provide 10 g/d of leucine (~125 g/d of dietary protein) with a minimum of 2.5 g of leucine at each of three meals (5,33). In order to maintain equal energy intake and minimize postprandial insulin response, protein was increased proportional to the reduction in dietary carbohydrates.
In comparisons with subjects following the USDA Food Guide Pyramid, subjects consuming the protein-rich diets lost more weight and were more effective in correcting body composition during weight loss (5,33). Consumption of the protein-rich diet resulted in greater loss of body fat and attenuated loss of lean tissue consistent with a protein sparing mechanism for leucine.

We also observed changes in glucose regulations with obese subjects during weight loss (34). Subjects consuming the high carbohydrate diet (ratio of carbohydrates:protein >3.5) maintained blood glucose within the normal physiological range; however, they had a progressive decline in fasting blood glucose and increase in postprandial insulin response during the 10-wk study. These data suggest that obese subjects challenged with a high carbohydrate diet exhibit a progressive decline in glycemic control. Subjects consuming the high protein diet (carbohydrate: protein <1.5) maintained stable blood glucose with minimal changes in blood glucose or insulin from fasting to meal periods.

Similar findings have been reported by other research groups. Diets with total protein intake >1.5 g · kg⁻¹ · d⁻¹ and carbohydrate intake <150 g/d increased weight loss (1–3,6), increased loss of body fat (2,3), attenuated loss of lean tissue (1,3,7), improved glycemic control (1,3,34), reduced serum triacylglyceride levels (5,6,33,35), and reduced blood pressure (36). These diets show efficacy in catabolic conditions, such as weight loss, resulting in these clinical outcomes. The spectrum of metabolic changes seen with weight loss also have specificity for the condition known as the Metabolic Syndrome (9,36,37).

### Protein-rich diets for treatment of the metabolic syndrome

Metabolic Syndrome or Syndrome X is a chronic disease affecting >1 in 5 adults in the U.S. It is a condition defined by glucose intolerance and compensatory hyperinsulinemia that is often observed with obesity. Specific criteria for screening and diagnosis of Metabolic Syndrome are elevated fasting blood glucose, elevated triglycerides, low HDL, abdominal obesity, and hypertension (Table 1). Presence of three of these characteristics is diagnostic for Metabolic Syndrome and highly predictive of risk for type 2 diabetes mellitus and coronary heart disease (36).

Although Metabolic Syndrome is often associated with obesity, the defining characteristic is abnormal glycemic control observed as glucose intolerance and insulin resistance (36). Stability of blood glucose requires balance between hepatic glucose release and peripheral glucose disposal. The liver regulates the rate of glucose appearance in blood by balancing absorption of dietary glucose with endogenous production of glucose from gluconeogenesis and glycogen breakdown (38,39). Use of blood glucose by peripheral tissues occurs through intracellular leucine concentration also serves as a primary regulator of the branched-chain ketoacid dehydrogenase (BCKD), a potent inhibitor of the BCKD.

### Impact of amino acids on glycemic control

Interactions of amino acids with carbohydrate metabolism have been recognized for years; however, research literature is unclear whether dietary protein has a positive or negative impact on glycemic control. Some reports suggest that amino acids increase fasting blood glucose (43–45), cause hyperinsulinemia (44), inhibit peripheral insulin action (44,45), and reduce glucose transport (44). By contrast, diets high in protein and low in carbohydrates reduce postprandial glucose and insulin and appear to stabilize blood glucose for individuals with type 2 diabetes (46) or obesity (1,3,34).

Dietary amino acids contribute to glucose homeostasis through hepatic glucose production. Jungs et al. (47) reported that amino acids serve as a primary fuel for the liver and the primary carbon source for hepatic gluconeogenesis. Other investigators found that gluconeogenesis provides >70% of fasting hepatic glucose release, with amino acids serving as the principal carbon source (38,42). Estimates of the contribution of amino acid carbon from 1 g of dietary protein to de novo glucose synthesis range from 0.6 to 0.7 g of glucose (46,48).

In addition to the direct conversion of amino acid carbon to gluconeogenesis precursors, there is also the contribution of the BCAA to glucose recycling via the glucose-alanine cycle (15,49). There is a continuous flux of BCAA from visceral tissues through the blood to skeletal muscle where transamination of the BCAA provides the amino group for production of alanine from pyruvate with a corresponding movement of alanine from muscle to liver to support hepatic gluconeogenesis. Although the impact of the glucose-alanine cycle has been debated, Ahlborg et al. (49) reported that alanine accounted for 40% of endogenous glucose production during prolonged exercise. Under normal conditions, alanine arising from BCAA nitrogen likely accounts for ~25% of gluconeogenesis from amino acids (8). These studies provide evidence for the linkage between dietary protein and glucose homeostasis.

Beyond roles of amino acids as direct carbon substrates for gluconeogenesis, leucine serves as a metabolic signal for fuel choices. As discussed above, leucine stimulates muscle protein synthesis through modulation of downstream elements of the insulin/Pi3-kinase signal pathway (Fig. 1). Parallel with mTOR actions on translational initiation factors, mTOR stimulates upstream phosphorylation of IRS-1 reducing Pi3-kinase activity (12,13). The significance of this feedback loop to the insulin receptor is unknown. We have shown that modulation of the Pi3-kinase activity does not alter glucose uptake into muscle tissue, leading us to propose that mTOR phosphorylation of IRS-1 is a component of normal feedback regulation perhaps limiting the duration of the insulin signal (13).

Intracellular leucine concentration also serves as a primary regulator of the branched-chain ketoacid dehydrogenase (BCKD), the rate-limiting step in oxidation of the BCAA (16). An increase in leucine increases the concentration of its ketoanalogue α-ketoisocaprate, a potent inhibitor of the BCKD.

### Table 1

<table>
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<th>Criteria for Screening and Diagnosis of Metabolic Syndrome¹</th>
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<td><strong>Fasting blood glucose</strong></td>
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<td><strong>Serum HDL cholesterol</strong></td>
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<td><strong>Waist circumference</strong></td>
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¹ Presence of three or more of these symptoms is diagnostic (36).
kinase that is responsible for inactivation of the BCKD by phosphorylation. Inhibition of the BCKD kinase results in dephosphorylation and activation of the BCKD. The BCKD stimulates decarboxylation of the three BCAAs and commits them to oxidation. At the same time, the increase in leucine concentration also inhibits pyruvate dehydrogenase–limiting pyruvate oxidation (50). Thus, when intracellular levels of leucine are elevated, muscles use glucose derived from either the blood or glycogen stores as a glycolytic fuel and then trap the pyruvate carbon as alanine via transamination with amino acid nitrogen derived from BCAA. These mechanisms appear to be particularly important during periods of low energy intake or endurance exercise when BCAAs are increased in muscle, insulin is low, and sparing of blood glucose is important (8,14,22).

The relative importance of reducing dietary carbohydrates versus increased dietary protein for glycemic control is difficult to assess using complete diets. Both carbohydrates and protein are known to affect stability of blood glucose (51–53). Diets high in carbohydrates increase the rate of appearance of glucose into the blood and inhibit gluconeogenesis (48,54). These diets reduce the role of gluconeogenesis in management of fasting blood glucose and shift glycemic regulation to glycogenolysis and glycogen stores (48,54). Opposite of this regulation, diets with reduced carbohydrates and increased protein increase rates of gluconeogenesis (43,48,54). Rossetti et al. (43) reported that increases in dietary protein were essential for control of protein synthesis, resulting in maintenance of muscle protein loss and enhanced glycemic control. Specific mechanisms to explain each of the observed outcomes remain to be fully elucidated.

We suggest that a key to understanding these results is that protein is hyperinsulinemic and hyperglycemic. In summary, use of diets with increased protein and reduced carbohydrates appears to enhance weight loss with increased loss of body fat and reduced loss of lean body mass. Beneficial effects of high-protein diets appear to be associated with sparing of muscle protein loss and enhanced glycemic control. Specific mechanisms to explain each of the observed outcomes remain to be fully elucidated. We suggest that a key to understanding the relationship between dietary protein and carbohydrates is the relationship between the intakes of leucine and glucose. Leucine is now known to interact with the insulin-signaling pathway with apparent modulation of the downstream signal for control of protein synthesis, resulting in maintenance of muscle protein during periods of restricted energy intake. Leucine also appears to modulate the insulin signal and glucose use by skeletal muscle. While total protein is important in providing substrates for gluconeogenesis, leucine appears to regulate oxidative use of glucose by skeletal muscle through stimulation of glucose recycling via the glucose-alanine cycle. These mechanisms appear to provide a stable glucose environment with low insulin responses during energy-restricted periods.

LITERATURE CITED

Commentary

**Dietary Guidelines should reflect new understandings about adult protein needs**
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**Abstract**

*Dietary Guidelines for Americans* provide nutrition advice aimed at promoting healthy dietary choices for life-long health and reducing risk of chronic diseases. With the advancing age of the population, the 2010 *Dietary Guidelines* confront increasing risks for age-related problems of obesity, osteoporosis, type 2 diabetes, Metabolic Syndrome, heart disease, and sarcopenia. New research demonstrates that the meal distribution and amount of protein are important in maintaining body composition, bone health and glucose homeostasis. This editorial reviews the benefits of dietary protein for adult health, addresses omissions in current nutrition guidelines, and offers concepts for improving the *Dietary Guidelines*.

**New concepts about protein for the Dietary Guidelines**

- Protein is a critical part of the adult diet
- Protein needs are proportional to body weight; NOT energy intake
- Adult protein utilization is a function of intake at individual meals
- Most adults benefit from protein intakes above the minimum RDA

The developing controversy about *Dietary Guidelines for protein stems from current perceptions that protein intakes above minimum requirements have no benefit and may pose long-term health risks. These beliefs are largely based on assumptions and extrapolations with little foundation in nutrition science. Diets with increased protein have now been shown to improve adult health with benefits for treatment or prevention of obesity, osteoporosis, type 2 diabetes, Metabolic Syndrome, heart disease, and sarcopenia [1-4]. This editorial argues that we need *Dietary Guidelines* that recognize these benefits and emphasize the right amounts of protein at specific meals.

Current perceptions are that protein is an expensive nutrient with limitations in the food supply and are reinforced by outcome measures that are based on strict cost/benefit approaches to diet formulation. This concept stems from animal science goals to maximize growth with the least expensive foodstuff. Animal feeding protocols focus on providing cheap carbohydrates as the primary energy source and limiting dietary protein to a substrate role for building new proteins. These measures are based on the body's ability to capture dietary nitrogen as body protein. Such thinking translates easily to childhood nutrition where growth and nitrogen accumulation are simple outcome measurements to confirm adequate dietary protein to maintain growth within percentile standards. Even measures of protein quality are derived from growth (Protein Efficiency Ratio: PER) and nitrogen balance (Net Pro-
tein Utilization: NPU) evaluated under conditions of limited protein intake [5]. With this history, dietary guidelines for protein evolved to provide only the minimum RDA.

During the past decade a growing body of research reveals that dietary protein intakes above the RDA are beneficial in maintaining muscle function and mobility [6] and in the treatment of diseases including obesity, osteoporosis, type 2 diabetes (T2DM), Metabolic Syndrome (MetS), heart disease, and sarcopenia [1-4]. The new research establishes health benefits and provides molecular evidence of numerous metabolic outcomes associated with protein intake or amino acid metabolism that are not reflected in the traditional measure of nitrogen balance. These outcomes include cell signaling via leucine [7,8], satiety [9,10], thermogenesis [11], and glycemic control [12,13]. The dietary protein necessary to optimize each of these metabolic outcomes is not reflected in measures of nitrogen balance and is not presented within the current concept of the minimum RDA. So what is known and what is missing in current Dietary Guidelines?

Current Status and Errors of Omission
Criteria for protein requirements are based on providing the minimum essential amino acids (EAA) necessary as building blocks for new protein structures [5]. The fundamental philosophy underpinning the RDA is that once substrate requirements for EAA are met then the need for protein is satisfied. Extension of this philosophy implies that any additional amino acids beyond the minimum RDA are unnecessary and have no nutritional value.

This concept of substrate adequacy is evaluated by short-term nitrogen retention. Titration of amino acids into the diet from protein-free to surfeit intakes produces an almost linear response in nitrogen balance from negative to positive. Nitrogen balance (i.e. intake = excretion) is assumed to reflect an Estimated Average Requirement (EAR ~0.66 g/kg/d)[14]. This EAR plus a safety factor is the current RDA (0.8 g/kg/d) defined as "the minimum daily needs for protein to maintain short-term nitrogen balance in healthy people with moderate physical activity" [14].

At the inflection point for nitrogen balance, plasma concentrations of EAA rise rapidly stimulating amino acid oxidation [5] and this is taken as confirmation that nitrogen balance provides a measure of protein efficiency. The increase in plasma amino acids is thought to represent saturation of substrate needs (i.e. charging of tRNAs) and any additional amino acids are degraded by oxidation to energy. Amino acid oxidation serves to confirm nitrogen balance as a measure of protein efficiency. Protein intakes above the inflection point in nitrogen balance or amino acid oxidation are considered to reflect inefficient utiliza-

Another major flaw in the Dietary Guidelines is the failure to recognize that dietary protein needs are inversely proportional to energy intake [15]. Current guidelines present protein needs as a percentage of energy in proportion to carbohydrates and fats. For example, MyPyramid represents the macronutrient goals as 55% of energy from carbohydrates, 30% from fats, and 15% from protein. At high energy intakes this balance of macronutrients is adequate. A 70 kg adult with energy intake of 2500 kcal/day would achieve a daily intake of 93 g of protein which is safely above the minimum RDA requirement of 56 g/day (i.e. 0.8 g/kg). However, if energy intake is reduced for weight management or during aging recommending protein as a percentage of energy is a serious error and potentially harmful. During weight loss, total daily energy intake is often below 1400 kcal/day. If the protein goal is represented as 15% of energy intake, daily protein intake is limiting at only 52 g. Protein needs are a function of lean tissue mass and must increase as a percentage of low energy diets.

The Food and Nutrition Board recognized the potential for biological diversity and individual choice with the DRI for macronutrients and created an Acceptable Macronutrient Distribution Range (AMDR)[14]. The AMDR for protein provides a minimum RDA intake of 0.8 g/kg with a range up to at least 2.5 g/kg without any identifiable Upper Limit risk. The AMDR range was unfortunately converted into percentage of energy intakes (10% to 35% of energy) to be consistent with guidelines for carbohydrates and fat. While this provides consistency for presentation of nutrient guidelines, presenting protein as a percent of energy reduces the apparent significance of dietary protein to that of a minor energy source. This is a critical conceptual issue for Dietary Guidelines. Consumers must understand that absolute protein requirements (grams per day) relate to body weight and remain virtually constant across all energy intakes. If protein recommendations are maintained as an indirect relationship with energy intake (10% to 35% of energy), then Dietary Guidelines must emphasize that protein needs increase by approximately 1% for every 100 kcal decrease in energy intake below 2000 kcal/day.

Another error of omission in the Dietary Guidelines relates to recognition that the efficiency of protein utilization decreases throughout adult life [6]. During aging, there is an increase in the requirement for EAA to produce a posi-
tive response in muscle protein synthesis [16,17]. The need for total protein may not change, but the effectiveness of amino acids to stimulate muscle (and probably bone) protein metabolism decreases requiring either more total protein or greater nutrient density of EAA/total protein (i.e. protein quality). The change in efficiency of EAA use appears to be associated with the loss of anabolic drive for development of lean tissue [18]. During growth, the body has a high metabolic priority for structural development of muscle and bone driven by anabolic hormones including insulin, growth hormone, IGF-1 and steroid hormones. Further, physical activity has a positive effect on the efficiency of use of amino acids [19]. Muscle protein synthesis is stimulated by stretching and resistance activity. The converse is also true; a sedentary lifestyle reduces the efficiency of EAA use. After approximately age 30 y, the anabolic drive is lost; basal levels of hormones become largely ineffective in stimulating protein synthesis in structural tissues; and diet quality and physical activity become the limiting factors for maintaining optimal protein turnover for repair, remodeling, and recovery.

In summary, omissions in current understanding of dietary protein needs are that 1) nitrogen balance and amino acid oxidation are only useful for defining minimum protein requirements and not optimum amino acid needs, 2) protein requirement is proportional to body weight and inversely proportional to energy intake, and 3) adults need more EAA than children to maintain the efficiency of protein turnover in structural tissues.

**New knowledge about protein**

Protein and amino acids contribute to multiple metabolic roles beyond simple substrates for protein synthesis. Dietary protein influences cell signaling, satiety, thermogenesis and glycemic regulations and each of these roles is initiated by increases in plasma and intracellular amino acid concentrations. These metabolic outcomes only become important with intakes above the minimum RDA. Using current measures of nitrogen balance and amino acid oxidation as the only criteria for protein requirements, these metabolic outcomes are rejected out-of-hand as inefficient and wasteful. A more logical view is that these new metabolic outcomes provide the basis for the AMDR and provide for individualization of dietary choice. Individuals can design healthy and adequate diets around the minimum RDA to prevent deficiency or design diets around higher levels of protein with additional health benefits.

Mechanisms for these metabolic outcomes are being unraveled and the effects appear to relate to the protein at each meal [20,21]. Current dietary guidelines focused on the RDA minimize the importance of protein as a central part of every meal and produce meal patterns with over 65% of protein consumed in a single large meal after 6:30 pm [22]. Most adults consume less than 10 g of protein at breakfast [23,24] (Figure 1). In children and young adults, uneven meal distribution of protein appears not to adversely affect growth. The anabolic drive maintains high efficiency of protein use for nitrogen retention even when daily protein is consumed as a single large meal. However in older adults, the quantity and quality of protein at individual meals is important. Adults require a minimum of 15 g of EAA or at least 30 g of total protein to fully stimulate skeletal muscle protein synthesis [21,25]. This response appears to be determined by the EAA leucine which serves as a critical signal for triggering initiation of

![Figure 1](image-url)

**Figure 1**

**Protein distribution at meals.** A) Ingestion of 90 grams of protein, distributed evenly at 3 meals. B) Ingestion of 90 grams of proteins unevenly distributed throughout the day. Stimulating muscle protein synthesis to a maximal extent during the meals shown in Figure 1A is more likely to provide a greater 24 hour protein anabolic response than the unequal protein distribution in Figure 1B. (Adapted from Paddon-Jones & Rasmussen Curr Opin Clin Nutr Metab Care 2009, 12: 86–90.)
muscle protein synthesis. Leucine has been well characterized as a unique regulator of the insulin-mTOR signal pathway controlling synthesis of muscle proteins [7,8]. In children and young adults, this signal pathway is regulated by insulin and dietary energy while leucine regulates the pathway in adults [26]. Current dietary patterns that provide adequate protein or leucine at only one meal produce an anabolic response only after that meal (Figure 1). This is a critical factor for protection of lean tissues during weight loss or to prevent age-related sarcopenia and osteoporosis.

The meal content of protein is also a key factor for satiety and appetite regulation [9,10]. Protein has greater satiety value than either carbohydrates or fats and reduces food intake at subsequent meals [27]. Studies of energy regulation for weight management show that replacing carbohydrates with protein reduces daily energy intake by ~200 kcal [9]. The mechanism for this satiety effect may be mediated by intestinal hormones or by reducing peak post-prandial insulin response. While the mechanism remains to be elucidated, it is clear that the improved satiety response requires >30 g of protein at a meal and that breakfast has the greatest impact on total daily energy intake [27]. As with protein turnover in muscle and bone, limiting protein intake to a single large meal late in the day reduces the satiety benefits of dietary protein [22].

The most unequivocal evidence for the benefit of increased dietary protein is derived from studies of weight management [1,28,29]. Diets with increased protein have been shown to be highly beneficial during weight loss because of their ability to correct body composition and increase satiety and thermogenesis. Higher protein diets increase loss of body weight and body fat and attenuate loss of lean tissue when compared with commonly recommended high carbohydrate low fat low protein diets [28,30]. Clearly, the major factors accounting for weight loss are the magnitude of energy restriction and individual compliance. Any diet can produce weight loss. However, long-term success with weight loss relates to maintenance of metabolically active lean tissues and research has proven that higher protein diets protect muscle and bone during weight loss. Use of conventional high carbohydrate, low fat, low protein diets results in 30% to 40% loss of lean tissue mass. Use of higher protein diets reduces lean tissue loss to <15% and when combined with exercise can halt loss of lean tissue during weight loss [30-32]. Studies also show that moderate protein diets have better long-term compliance.

The effects of protein for maintaining lean tissues appear to translate into health benefits during aging where progressive loss of structural strength and mobility are critical factors. Osteoporosis and sarcopenia have emerged as major issues during aging [2,3]. Prevention of osteoporosis is associated with physical activity and dietary calcium and protein [3]. The efficacy of calcium and protein are interrelated [3]. Calcium supplements are largely ineffective for remodeling of bone matrix if protein is limiting. Positive effects of calcium appear to require intakes of protein >1.2 g/kg to have beneficial effects. The long-held belief that increased dietary protein could cause bone loss as reflected in increase urinary calcium is incorrect [33] and protein is now recognized to increase intestinal calcium absorption in addition to enhancing bone matrix turnover [34].

Similar results have been observed with studies of muscle health in elderly where the efficiency of EAA use is reduced [16,17]. The level of EAA required to stimulate muscle protein synthesis is increased in part due to reduced anabolic stimulus of hormones. Here again it is important to distinguish the difference between outcome measures of muscle protein metabolism versus nitrogen balance. Long-term prospective outcomes with protein supplementation and muscle function are not available. However cross-sectional studies support the idea that earlier in higher percentiles of protein intake have less age-related decline in lean tissue mass [35].

Emerging health concerns relate to macronutrient choices for T2DM and MetS [4]. These conditions are characterized by dysregulation of glucose metabolism and have raised new questions about the quantity and quality of carbohydrates in the diet. Extensive research about types of carbohydrates and glycemic index have emerged but evidence is convincing that reduction in total dietary carbohydrates to less than 40% of total energy is the most effective way to improve glycemic regulations in T2DM and MetS [4].

Early research with MetS evaluated reducing dietary carbohydrates with fats [36]. While increasing dietary fats improved glycemic control and reduced cardiovascular disease (CVD) risk, the prospect of increasing dietary fat remains controversial. Replacement of carbohydrates with protein improves glycemic control measured as reduced post-prandial hyperinsulinemia [37] and in T2DM corrects hyperglycemia and HbA1c [13]. Equally important, reduced carbohydrate diets have decreased TAG, increased HDL and increased LDL particle size (i.e. LDL-C/ApoB) improving the dyslipidemia commonly associated with T2DM and MetS [4]. These conditions are 4-times more important for heart disease and all cause mortality than elevated cholesterol or LDL concentration [38].

**New understandings about protein for the Dietary Guidelines**

- **Protein is a critical part of the adult diet**

Protein should be a central part of a complete diet for adults. While physical growth occurs only for a brief
period of life, the need to repair and remodel muscle and bone continues throughout life. Maintaining the health of muscle and bone is an essential part of the aging process and critical to maintain mobility, health and the active tissues of our body. Protein needs become more important during periods of reduced food intake such as weight loss or during periods of recovery after illness or during aging.

- **Protein needs are proportional to body weight; NOT energy intake**

Protein needs for adults relate to body weight. Dietary protein need is often presented as a percentage of energy intake. The DRIs represent the acceptable protein range as 10% to 35% of total energy. However, protein needs are constant across all energy intakes. So at low energy intakes, protein needs to be a higher percentage of total calories and at high energy intakes protein can be reduced as a percentage of total calories. In general, dietary protein should be established first in any diet in proportion to body weight and then carbohydrates and fats added determined by energy needs.

- **Optimal adult protein use is a function of intake at individual meals**

Protein is an important part of good nutrition at every meal. Vitamins and minerals can fulfill nutrient needs on a once-per-day basis but for protein the body has no ability to store a daily supply. To maintain healthy muscles and bones for adults, at least 30 g of protein should be consumed at more than one meal. Breakfast is an important meal for dietary protein because the body is in a catabolic state after an overnight fast. A meal with at least 30 g of protein is required to initiate repletion of body proteins. Protein at breakfast is also critical for regulation of appetite and daily food intake.

- **Most adults benefit from protein intakes above the minimum RDA**

Aging populations confront increasing incidence of obesity, osteoporosis, type 2 diabetes, Metabolic Syndrome, heart disease, and sarcopenia which have raised new questions about dietary ratios of carbohydrates, fats, and protein for life-long health. The RDA represents the minimum daily intake for active healthy adults. For most adults, replacing some dietary carbohydrates with protein will help to maintain body composition and mobility, improve blood lipids and lipoproteins, and help to control food intake.

**Competing interests**

DKL has received honorarium for participation in speaker bureaus for the National Dairy Council (NDC) and National Cattlemen’s Beef Association (NCBA), serves on the Research Advisory Board for the Egg Nutrition Center (ENC), and has research funding from NDC and ENC.

**References**

23. USDA/NHANES: [http://www.ars.usda.gov/SP2UserFiles/Place/12355200/pdf/Table_1_BIA.pdf](http://www.ars.usda.gov/SP2UserFiles/Place/12355200/pdf/Table_1_BIA.pdf).
24. USDA/NHANES: [http://www.ars.usda.gov/SP2UserFiles/Place/12355200/pdf/Table_9_BIA.pdf](http://www.ars.usda.gov/SP2UserFiles/Place/12355200/pdf/Table_9_BIA.pdf).