The Emerging Role of Dairy Proteins and Bioactive Peptides in Nutrition and Health

Dietary Protein Impact on Glycemic Control during Weight Loss[1]

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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

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TABLE 1

Leucine and BCAA content of foods[1]

	Leucine	BCAA
Whey protein isolate	14%	26%
Milk protein	10%	21%
Egg protein	8.5%	20%
Muscle protein	8%	18%
Soy protein isolate	8%	18%
Wheat protein	7%	15%

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

maintain short-term stability of body protein is ~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 ~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 $p70^{S6}$ 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 ~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 predominate 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 μU/mL (35–140 pmol/L).

During periods of food intake and absorption of exogeneous 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

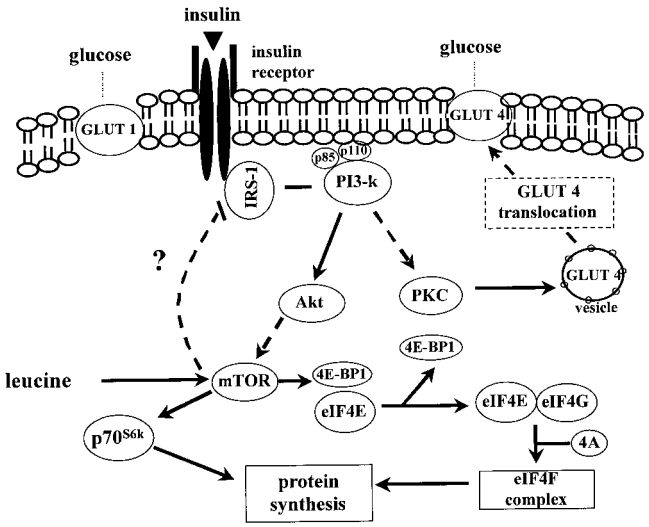

FIGURE 1 Insulin signaling cascade. GLUT1, insulin independent glucose transporter; PKC, protein kinase C; eIF, translational initiation factors.

[3] Abbreviations used: AUC, area under the curve; BCKAD, branched-chain ketoacid dehydrogenase; 4E-BP1, inhibitory binding protein; EGP, endogenous glucose production; GLUT4, insulin dependent glucose transporter; IRS-1, insulin receptor substrate; mTOR, mammalian target of rapamycin; PI3-K, phosphatidylinositol-3 kinase; Ra, rate of appearance.

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 adult 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

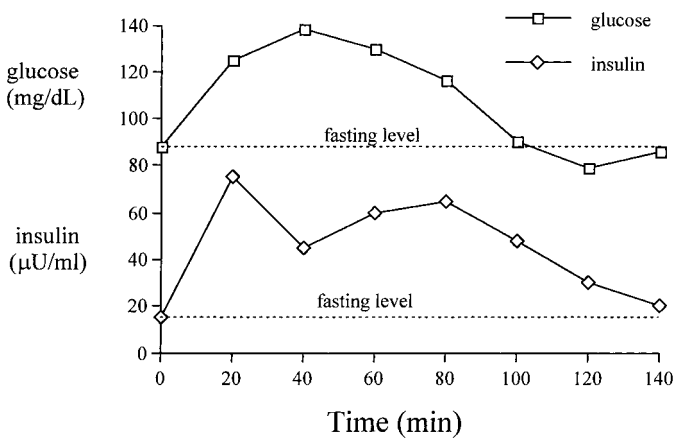

FIGURE 2 Theoretical glucose and insulin curves for responses to a 400 kcal (1675 kJ) breakfast meal.

(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 glucose homeostasis 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 BCAA, 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 a high protein 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-

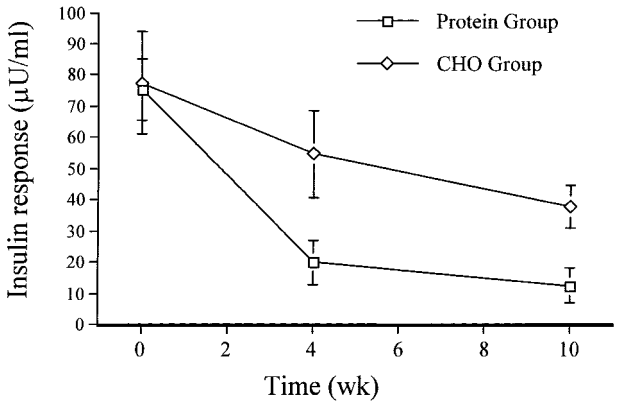

FIGURE 3 Curves represent 2-h postprandial insulin responses to a 400 kcal (1675 kJ) test meal in overweight women assigned to either moderate protein (Protein Group) or a high carbohydrate (CHO Group) weight loss diets. Both diets provided 1700 kcal/d (7117 kJ/d) (8). The insulin response was determined as the 2-h postprandial value minus the fasting value for each subject. Values represent means ± SEM, *n* = 5 (Layman, unpublished).

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 $p70^{S6}$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 pyurvate 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.

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