

Leucine Regulates Translation Initiation of Protein Synthesis in Skeletal Muscle after Exercise^{1,2}

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ABSTRACT High-performance physical activity and postexercise recovery lead to significant changes in amino acid and protein metabolism in skeletal muscle. Central to these changes is an increase in the metabolism of the BCAA leucine. During exercise, muscle protein synthesis decreases together with a net increase in protein degradation and stimulation of BCAA oxidation. The decrease in protein synthesis is associated with inhibition of translation initiation factors 4E and 4G and ribosomal protein S6 under regulatory controls of intracellular insulin signaling and leucine concentrations. BCAA oxidation increases through activation of the branched-chain α -keto acid dehydrogenase (BCKDH). BCKDH activity increases with exercise, reducing plasma and intracellular leucine concentrations. After exercise, recovery of muscle protein synthesis requires dietary protein or BCAA to increase tissue levels of leucine in order to release the inhibition of the initiation factor 4 complex through activation of the protein kinase mammalian target of rapamycin (mTOR). Leucine's effect on mTOR is synergistic with insulin via the phosphoinositol 3-kinase signaling pathway. Together, insulin and leucine allow skeletal muscle to coordinate protein synthesis with physiological state and dietary intake. *J. Nutr.* 136: 533S–S537, 2006.

KEY WORDS: • *insulin* • *mTOR* • *leucine* • *muscle* • *exercise*

Muscle protein undergoes constant change and remodeling through synthesis of new proteins and breakdown of existing proteins. Together, these processes are called protein turnover and produce muscle growth or hypertrophy when synthesis is greater than breakdown and muscle wasting when synthesis is less than breakdown. For nongrowing adults, maintenance of constant muscle mass requires zero daily balance between synthesis and breakdown. During the course of a day, the comparative ratios of protein synthesis to breakdown change constantly. After a meal as nutrients are absorbed, the rate of protein synthesis increases. The rate of protein breakdown also rises but to a lesser extent, resulting in a net positive balance of protein turnover (Fig. 1). After an overnight period without food, protein synthesis decreases by 15 to 30% depending on the length of the fast. Protein breakdown changes less, resulting in a net catabolic period (Fig. 1). The catabolic period after an overnight fast continues until adequate energy and amino acids

are available to stimulate protein synthesis. These short-term changes in the regulation of protein turnover appear to be produced by changes in the initiation phase of translation control of protein synthesis. Key nutrition factors appear to be energy status of the cell and intracellular concentration of the BCAA leucine (1,2).

Exercise-induced changes in protein turnover

Exercise produces diverse changes in amino acid metabolism and protein turnover in skeletal muscle. Acute changes are driven by energy needs and amino acid availability, whereas long-term changes allow for adaptation of proteins for structure and performance (3,4). Acute changes in amino acid metabolism caused by exercise are largely catabolic with net negative balance between the rates of protein synthesis and protein breakdown and an increase in the rate of amino acid oxidation. The magnitudes of these catabolic processes are determined by the type of exercise. Although acute effects of exercise are catabolic, exercise clearly does not cause muscle wasting; instead, regular exercise is essential to optimize muscle growth and hypertrophy. Thus, exercise requires a sequence of metabolic adjustments from the catabolic period of exercise to the anabolic period of recovery.

Exhaustive endurance exercise inhibits muscle protein synthesis with the magnitude of the depression related to the intensity and duration of the activity (5–7). Similar to changes during fasting, protein breakdown is higher than protein synthesis during exercise, resulting in a net catabolic period (Fig. 1). The time course of the changes in protein synthesis

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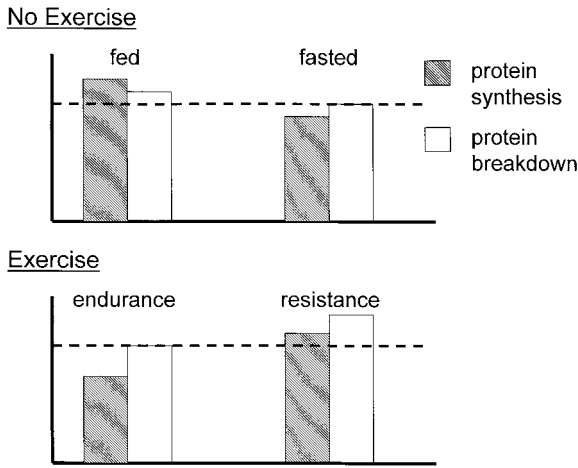


FIGURE 1 Skeletal muscle protein turnover illustrates the balance between protein synthesis (*shaded bars*) and protein breakdown (*open bars*) during different physiological conditions, including the anabolic period after a meal or the catabolic period during an overnight fast. Exercise examples represent exhaustive endurance exercise and a prolonged bout of resistance exercise.

and breakdown are unknown, largely due to limitations in making measurements during exercise. Protein turnover remains negative until adequate dietary protein and energy are available for recovery.

Resistance exercise produces a pattern of changes in protein turnover that are somewhat different. At the end of exhaustive resistance exercise, protein synthesis is increased (8,9). Yet, a single bout of resistance exercise is still catabolic due to increases in protein breakdown (Fig. 1). Similar to endurance exercise, skeletal muscle remains in negative nitrogen balance after resistance exercise until adequate protein and energy are available for recovery (10). The molecular mechanisms determining the key regulation of protein synthesis under these conditions place leucine in a central role in the initiation of translation.

Metabolic roles of leucine in skeletal muscle

Exercise stimulates changes in protein and amino acid metabolism. Amino acid metabolism in skeletal muscle is limited to six amino acids (glutamate, aspartate, asparagine, and the three BCAAs). Among these amino acids, the most noteworthy effects have been observed with the BCAA leucine (3,7,11). Leucine participates in numerous metabolic processes (2), including its obvious role as a constituent of protein. Of more direct interest to the present discussion, leucine also functions as (1) a critical regulator of translation initiation of protein synthesis, (2) a modulator of the insulin phosphoinositol 3-kinase (PI3-kinase)⁴ signal cascade, and (3) a nitrogen donor for muscle production of alanine and glutamine. The potential for leucine to impact on translation initiation, insulin signaling, and the production of alanine and glutamine depends on its intracellular concentrations. The intracellular leucine concentration represents a balance between the rates of appearance of leucine from plasma uptake and intracellular protein breakdown and the rates of removal of leucine through intracellular amino acid oxidation and protein synthesis.

Leucine is unique among amino acids for its regulatory roles in metabolism, including translational control of protein synthesis (1) and glycemic regulation (12) (Fig. 2). This review focuses on the role of leucine in the regulation of skeletal muscle protein synthesis through initiation factors 4E (eIF4E) and 4G (eIF4G) and ribosomal protein S6 (rpS6). Other aspects of metabolism sensitive to intracellular leucine concentrations include (1) the branched-chain α -keto acid dehydrogenase (BCKDH), the rate-limiting step in BCAA degradation (13); (2) pyruvate dehydrogenase, a key enzyme in glycolysis that controls pyruvate entry into the TCA cycle (14); (3) the insulin receptor substrate-1, the initial phosphorylation target of the insulin receptor; and (4) the pancreatic β -cell, in relation to insulin release (12). In total, these diverse metabolic roles allow leucine to influence directly the rate of muscle protein synthesis, insulin action, and glucose homeostasis.

The effects of leucine are, at least in part, associated with the absence of the branched-chain aminotransferase (BCAT) enzyme in liver (15). Absence of BCAT in liver results in an enriched supply of the three BCAAs channeled to skeletal muscle. Dietary BCAA reach the blood virtually unaltered from their levels in the diet; thus, leucine reaches peripheral tissues in direct proportion to its dietary intake. During exercise, there also is increased release of leucine from visceral tissues (liver and gut) and movement to skeletal muscle (16). This pattern of interorgan movement of amino acids provides for a continuous supply of leucine to skeletal muscle.

Constant influx of leucine to skeletal muscle is buffered by the presence of BCAT and BCKDH, the enzymes catalyzing the first two steps in BCAA degradation (15). BCKDH mediates the rate-limiting step in BCAA degradation; its activity is proportional to intracellular BCAA concentrations. In skeletal muscle, BCKDH is normally <20% active with great capacity to respond to increased BCAA influx. Thus, although the plasma levels of BCAA may change 2- to 3-fold after a meal, the changes in intracellular leucine concentrations are much smaller due to buffering by BCKDH (2,15).

Our initial interest in the role of leucine in postexercise recovery began with the observation in young athletes that plasma leucine concentration decreases dramatically at the end of exhaustive endurance exercise (17). We modeled this change in plasma leucine using rats and treadmill running. At the end of a 2-h exhaustive run, rats experienced a 25% decrease in the rate of muscle protein synthesis, compared with fasted controls receiving no exercise (18). These animals were provided with a series of recovery drinks to evaluate the impact of carbohydrate and protein on muscle protein synthesis

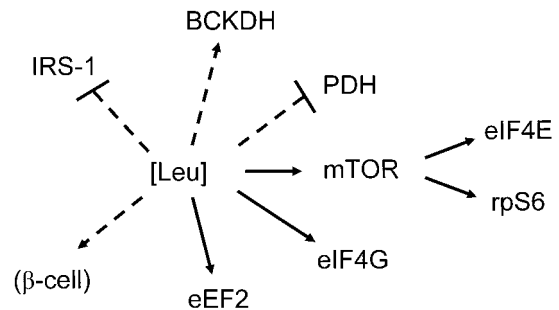


FIGURE 2 Intracellular leucine concentrations influence protein synthesis (*solid lines*), including translation initiation factors eIF4E, rpS6, and eIF4G and elongation factor eEF2, and energy metabolism (*dashed lines*) through BCKDH, pyruvate dehydrogenase (PDH), insulin receptor substrate-1 (IRS-1), and insulin release from the β -cell of the pancreas. Arrows indicate stimulation, and blocked lines indicate inhibition.

⁴ Abbreviations used: AMPK, AMP kinase; BCAT, branched-chain aminotransferase; BCKDH, branched-chain α -keto acid dehydrogenase; eIF4E, initiation factor 4E; eIF4G, initiation factor 4G; mTOR, mammalian target of rapamycin; PI3-kinase, phosphoinositol 3-kinase; PKB, protein kinase B; p70^{S6K}, 70-kD ribosomal protein S6 kinase; rpS6, ribosomal protein S6; tRNA, transfer RNA.

(18,19). An electrolyte drink containing glucose and sucrose increased blood glucose and insulin concentrations as well as muscle glycogen content but produced no recovery of muscle protein synthesis. However, a complete meal containing protein (or leucine alone) produced complete recovery of muscle protein synthesis within the first hour after exhaustive exercise. It is now clear that leucine stimulates muscle protein synthesis through the protein kinase mammalian target of rapamycin (mTOR) activating initiation factors eIF4E and rpS6 (2,20). Interestingly, plasma levels of leucine do not decrease after resistance exercise (21); likewise, rates of skeletal muscle protein synthesis do not decrease after resistance exercise compared with nonexercised controls (8,9). However, net protein balance remains negative after resistance exercise, as protein breakdown is greater than protein synthesis, and remains negative until dietary protein or leucine is ingested (22).

Translational control of muscle protein synthesis

Short-term regulation of protein synthesis is achieved at the level of translation, with primary emphasis on the assembly of the structural machinery for protein synthesis through a process known as initiation (1,23). The basic components for protein synthesis include the large and small ribosomal subunits (60S and 40s, respectively), mRNA coding for individual proteins, transfer RNA (tRNA) for individual amino acids, and more than a dozen catalytic proteins identified as eIFs. These initiation factors guide the assembly of the ribosome on the mRNA and are responsive to short-term changes in the availability of energy, amino acids, and growth factors. Initiation factors provide the cell with sensitivity to environmental factors, including changes in diet, such as leucine availability, and physical activity.

Among the catalytic peptides regulating protein synthesis, most attention has focused on initiation factors eIF2, the eIF4F complex, and rpS6. Initial assembly of a ribosome on mRNA requires activation of the 40S ribosomal subunit and preparation of the 5' cap end of the mRNA. Activation of the 40S subunit requires binding with eIF2, which carries a high energy molecule of GTP, and the initiator amino acid methionine (Met-tRNA). This complex of eIF2-GTP-Met-tRNA (known as the ternary complex) binds with the 40S subunit to form the 43S pre-initiation complex. Formation of the ternary complex is sensitive to cellular energy status and intracellular leucine concentrations. Formation of the 43S pre-initiation complex is an essential first step in the initiation sequence; however, eIF2 is seldom limiting in skeletal muscle (1,24).

Binding of the 43S pre-initiation complex to an mRNA is believed to be a rate-controlling step in translation initiation (1,24,25). This step is mediated by eIF4F, a three-subunit complex consisting of (1) eIF4E, a protein that binds to the 5' cap structure of the mRNA to be translated; (2) eIF4G, a peptide that serves as a scaffold to connect eIF4E and eIF4A with the mRNA and 40S subunit; and (3) eIF4A, a RNA helicase that functions to unwind secondary structure in the 5'-untranslated region of the mRNA. The eIF4F complex collectively serves to recognize, unfold, and guide the mRNA to the 43S pre-initiation complex.

Formation of the eIF4F complex is regulated through the availability of eIF4E for binding with eIF4G and the phosphorylation state of eIF4G. Availability of eIF4E for eIF4G is controlled by the eIF4E inhibitory binding protein 4E-BP1. The binding site for 4E-BP1 on eIF4E overlaps with the binding site for eIF4G, preventing formation of the eIF4E-eIF4G complex (26). Binding of 4E-BP1 to eIF4E is determined by the phosphorylation state of the binding protein. Phosphorylation of

4E-BP1 by the protein kinase mTOR reduces the affinity of 4E-BP1 for eIF4E (Fig. 3), allowing eIF4E to bind with eIF4G and form the active eIF4F complex. Activity of mTOR is sensitive to leucine concentration and stimulation through the PI3-kinase signal cascade.

Another downstream target of mTOR is the 70-kD rpS6 kinase (p70^{S6K}). p70^{S6K} is activated by phosphorylation from mTOR (1,24). Inhibiting mTOR prevents phosphorylation of the kinase and thus prevents the activation of p70^{S6K}. Activation of p70^{S6K} results in preferential translation of mRNAs that encode components of the protein synthesis mechanism, including the ribosomal proteins eIF4G, eukaryotic elongation factors (eEF1 and eEF2), and poly(A) binding protein. All of these proteins are involved in translation of mRNA to protein; thus, activation of rpS6 increases the cell's capacity for protein synthesis. Together, the effects of leucine concentration through mTOR activity on eIF4E and rpS6 influence both the rate of translation and the translation capacity in skeletal muscle.

Regulation of muscle protein synthesis via mTOR

Activation of mTOR is not fully understood but is influenced by multiple regulatory proteins, including the tuberous sclerosis complex (TSC1 and TSC2), Rheb, and AMP kinase (AMPK) (27–29) (Fig. 3). TSC1/TSC2 and Rheb are crucial regulators situated between protein kinase B (PKB) and mTOR. Rheb, a Ras-like GTPase, is a positive regulator of mTOR in vivo. The action of Rheb is opposed by the TSC1/TSC2 complex, which acts as a Rheb GTPase, promoting the conversion of Rheb-GTP to Rheb-GDP, inhibiting Rheb's positive effect on mTOR. TSC2 is sensitive to growth factors and energy (AMPK) but not to amino acids (30). In TSC2 knockout cells, amino acid deprivation still impairs mTOR signaling (31), suggesting that the primary site for leucine effects is downstream from TSC2, most likely through Rheb (29).

Energy status of the cell affects mTOR activity through the activity of AMPK. AMPK is widely regarded as an energy sensor. Under conditions of exercise, hypoxia, ischemia, heat shock, and low glucose, AMPK is activated by rising AMP levels and reduced glycogen content (32,33). Once AMPK is activated, it phosphorylates multiple substrates aimed at increasing intracellular ATP levels. AMPK also spares ATP by inhibiting the ATP-consuming process of protein synthesis.

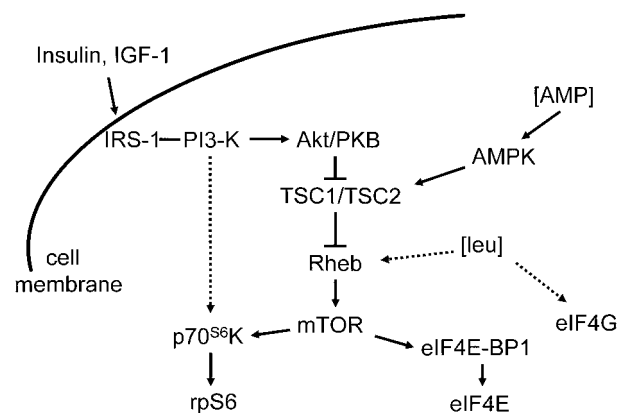


FIGURE 3 The metabolic signal cascade of PI3-kinase-PKB-mTOR serves to integrate physiological inputs from growth factors (insulin and IGF-1), the energy status of the cell (AMP concentrations), and intracellular leucine (leu) concentrations to regulate translation initiation at eIF4E, eIF4G, and rpS6.

Increased AMPK activity is associated with reduced eIF4E binding with eIF4G and increased 4E-BP1 inhibition of eIF4E (30). Smith et al. (31) proposed that the effects of AMPK on mTOR are mediated through TSC2. AMPK directly phosphorylates TSC2, increasing formation of the TSC1/TSC2 complex and inhibiting Rheb. Because AMPK is activated by exercise and a decreased ATP/AMP ratio, AMPK activation and its effects on the mTOR pathway appear to be a primary mechanism reducing protein synthesis after endurance exercise (30).

Leucine and mTOR-independent mechanisms

Although important, mTOR alone does not fully account for leucine stimulation of muscle protein synthesis. For instance, co-administration of leucine with the mTOR inhibitor rapamycin only partially inhibited the leucine-induced increase in protein synthesis (34). Furthermore, administration of leucine to diabetic rats increased muscle protein synthesis in the absence of increases in 4E-BP1 or p70^{S6K} phosphorylation (35). Likewise, rat hindlimbs perfused with leucine at food-deprived (1×) or superphysiologic (10×) concentrations of leucine demonstrate increases in protein synthesis in high vs. low leucine groups, even though signaling through mTOR and phosphorylation status of 4E-BP1 and p70^{S6K} were not different (36). Furthermore, there was a lack of correlation between leucine concentrations and 4E-BP1 binding with eIF4E and changes in eIF4G association with eIF4E (37). It is therefore likely that increased intracellular leucine concentrations stimulate protein synthesis via mTOR-dependent and mTOR independent pathways.

A known mechanism by which leucine stimulates protein synthesis independent of mTOR activity is through direct activation of eIF4G (Fig. 3). Bolster et al. (36) found that eIF4G phosphorylation was significantly increased in rats supplemented with leucine. They concluded that the increase in eIF4G phosphorylation increased the availability of eIF4G for eIF4E, thus increasing the formation of the eIF4E-eIF4G complex and protein synthesis independent of mTOR. These effects have been confirmed by Jefferson's group (38), who showed that even physiologically small increases in leucine concentrations increase eIF4G phosphorylation and protein synthesis compared to controls. These findings suggest that the amount of eIF4G available for formation of the eIF4G-eIF4E complex is limiting and that the release of a fraction of the total eIF4E from the eIF4E-4E-BP1 complex provides enough eIF4E to promote maximal formation of the eIF4G-eIF4E complex. Thus, leucine concentration is important for both availability of eIF4E and activation of eIF4G (Fig. 3).

Effect of exercise on translation initiation factors

Endurance exercise reduces the rate of muscle protein synthesis in proportion to the duration and intensity of activity (5–7). Exhaustive exercise or prolonged low frequency stimulation inhibits mTOR pathways, including inhibition of eIF4E and rpS6. After exercise, there is increased eIF4E binding to the inhibitor 4E-BP1 and reduced binding with the eIF4G initiation complex (18,30). Recent reports suggest the mechanism is due to increased activity of the AMPK (30). Exhaustive exercise decreases ATP, increases the concentration of AMP, and reduces glycogen concentration. Increased AMP and reduced glycogen stimulate AMPK, which leads to phosphorylation of TSC2, formation of the TSC1/TSC2 complex, and inhibition of Rheb and mTOR. Postexercise supplemental leucine increases intracellular leucine concentrations, which directly stimulates mTOR and eIF4G, allowing for recovery of

muscle protein synthesis (34,36). The combination of leucine plus carbohydrates appears to produce a synergistic effect on recovery, presumably through the combined effects of leucine on mTOR and insulin on PI3-kinase and PKB, resulting in reduced AMPK and TSC2 activity (18,19,30).

Resistance exercise produces increases in protein synthesis that are evident soon after exercise and persist for up to 48 h (22). This increase in protein synthesis appears to be mediated through PI3-kinase and PKB signaling, which are likely stimulated by growth factors such as insulin-like growth factor 1 (IGF-1) and myostatin (30). Studies using high-frequency stimulation in rats found increases in PKB, resulting in increased phosphorylation of TSC1 and reduced formation of the TSC1/TSC2 inhibitory complex, allowing for binding of Rheb with mTOR and activation of eIF4E and rpS6 (30) (Fig. 3). Furthermore, there is evidence for direct activation of rpS6 by PI3-kinase, which serves to increase the cellular capacity for protein synthesis after resistance exercise (30). While the combination of high-frequency stimulation and growth factors increases protein synthesis postexercise, synthesis is not fully stimulated and skeletal muscle remains catabolic without supplemental dietary leucine, either alone or as a part of protein or an amino acid mixture. Supplemental leucine allows for the muscle to achieve maximum protein synthesis and anabolic recovery (10,22).

In summary, exercise highlights the importance of the BCAA leucine in the regulation of muscle protein synthesis. After exercise, protein turnover is in negative balance—protein synthesis is slower than protein breakdown. This negative balance reflects inhibition of numerous components of translation initiation. For endurance exercise, energy demands increased AMPK, stimulating formation of the TSC1/TSC2 complex, which inhibits peptide initiation at mTOR. On the other hand, resistance exercise generally does not affect AMPK but increases total capacity for protein synthesis through PI3-kinase and PKB activation of rpS6. In both cases, recovery is apparently dependent on supplemental dietary leucine in order to increase the intracellular leucine concentration, which activates mTOR and the initiation factors eIF4E and eIF4G. At reduced intracellular leucine concentrations, eIF4G and 4E-BP1 are hypophosphorylated, formation of the eIF4E-eIF4G complex is inhibited, and protein synthesis is reduced. At slightly elevated intracellular leucine concentrations, 4E-BP1 becomes phosphorylated, while eIF4G remains hypophosphorylated, resulting in limited formation of the eIF4E-eIF4G complex and increased protein synthesis. Elevated intracellular leucine concentrations fully phosphorylate eIF4G, allowing for formation of the eIF4E-eIF4G complex with maximum stimulation of protein synthesis. Carbohydrates, nonessential amino acids, and other essential amino acids do not have stimulatory effects on protein synthesis when compared with leucine (18,39,40). However, in most cases, the combination of amino acid supplements with carbohydrates produces additive effects on the stimulation of the PI3-kinase and mTOR pathways, producing the maximum rates of protein synthesis during recovery.

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