control of muscle protein synthesis in response to exercise and amino acids

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abstract

there is a general consensus that resistance exercise and nutrition (especially amino acids) are the most effective interventions for maintaining skeletal muscle mass. the intracellular signaling pathways through the mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, are the most established mechanism for controlling muscle protein synthesis. acute bouts of resistance exercise and amino acid ingestion independently activate mTOR and its downstream targets that modulate protein translation initiation and elongation. although resistance exercise can modulate protein synthesis by endocrine regulation, such as the secretion of hormones and growth factors, one of the most recognized mechanisms for controlling muscle mass by resistance exercise involves mechanical tension. in regard to nutritional regulation, recent research indicates that intracellular amino acid availability, particularly that of leucine, may be a primary regulator of muscle protein synthesis following the ingestion of amino acids. the authors previously reported that leucine catabolism also has a significant impact on amino acid-induced protein synthesis. in contrast to established downstream molecular targets such as p70 S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding protein (4E-BP1), the upstream mediators, for regulating mTOR and protein synthesis in response to resistance exercise and amino acids, remain to be clarified. in this brief review, current progress regarding the intracellular mechanisms of muscle protein synthesis, in response to resistance exercise and amino acid ingestion, is discussed.

keywords: amino acids, exercise, mTOR, skeletal muscle, mechanical stimuli

introduction

the maintenance of skeletal muscle mass is very important for the prevention of lifestyle-related diseases and for quality of life. skeletal muscle mass is reduced by aging and physical inactivity. in general, lean muscle mass contributes up to 50% of total body weight in young adults, but declines with aging to ~25% at approximately 80 years of age. a decrease in skeletal muscle mass greatly impairs not only movement of the body, but also metabolic health, since muscles both serve as storage sites for energy for other tissues and consume excess energy.

the maintenance of skeletal muscle mass is primarily controlled by the balance between the rates of protein synthesis and protein breakdown. an increase in skeletal muscle mass results when the rate of protein synthesis is greater than the rate of protein degradation. the most widely recognized stimuli to enhance muscle protein synthesis are resistance exercise and nutrition. in this brief review, we discuss recent advances in the understanding of the cellular mechanisms that regulate muscle protein synthesis in response to resistance exercise and nutrients (amino acids).

regulation of mTOR signaling and protein synthesis

the mammalian target of rapamycin (mTOR) is a key regulator of protein synthesis in skeletal muscle (see Fig. 1). mTOR is a serine/threonine protein kinase of the phosphatidylinositol kinase-related family and is highly conserved from yeast to mammals. in eukaryotes, mTOR is found in two independently regulated, functionally distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 has four components in addition to mTOR: regulatory-associated protein of mTOR (raptor), mammalian lethal with SEC13 protein 8 (mLST8, also known as GβL), proline-rich Akt substrate 40 kDa (PRAS40), and DEP domain-containing mTOR-interacting protein (DEPTOR). raptor has many functions including regulation of mTORC1 assembly and recruiting kinase substrates. when activated, the mTOR component of mTORC1 phosphorylates both PRAS40 and DEPTOR, which weakens their association with the remainder of mTORC1 and promotes kinase activity. mTORC1 is the nutrient-responsive mediator of cell growth regulation. on the other hand, signaling by mTORC2 is not directly inhibited by rapamycin, and it seems unlikely that mTORC2 contributes to growth-regulatory effects. for the purposes of this review, the
role of mTORC1 in resistance exercise- and amino acid-induced activation of muscle protein synthesis is discussed.

Growth factor-dependent activation of mTORC1 has been intensively studied. The phosphatidylinositol-3 kinase (PI3K)/Akt (also called protein kinase B) pathway plays a critical role in the regulation of mTORC1 by growth factors. The binding of a growth factor to its receptor leads to activation of its intrinsic tyrosine kinase and autophosphorylation, which generates docking sites for insulin receptor substrates (IRSs). Phosphorylated IRSs then act as docking sites to recruit and activate PI3Ks, which generates phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 acts as a docking site for phosphoinositide-dependent kinase 1 (PDK1) and Akt, a serine/threonine kinase that is the central mediator of the PI3K pathway. Once activated, Akt stimulates protein synthesis by activating mTORC1 through the intermediate tuberous sclerosis 1/2 complex (TSC1/2). The phosphorylation of TSC2 by Akt results in the destabilization of TSC2 and disruption of its interaction with TSC1, leading to the activation of Ras-homolog enriched in brain (Rheb) and mTORC1. In addition to the PI3K/Akt pathway, Ras-extracellular regulated kinase (ERK) signaling has also been associated with the activation of mTORC1 in response to growth factors. ERK is activated upon Ras-
induced activation of mitogen-activated protein kinase (MEK), followed by the phosphorylation of TSC2.

mTORC1 regulates protein synthesis by activating the translation of a subset of mRNA. Translational regulatory protein p70 S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding protein (4E-BP1) are well-known substrates of mTORC1. The activation of p70S6K by mTORC1 promotes mRNA translation through several substrates, such as eukaryotic elongation factor 2 kinase (eEF2K), eukaryotic initiation factor 4B (eIF4B), and ribosomal protein S6. Dey et al. reported that low-load (30% of repetition maximum) high-volume resistance exercise is more effective for muscle protein synthesis than high-load low-volume exercise. Burd et al. reported that low-load (30% of repetition maximum) high-volume resistance exercise is more effective for muscle protein synthesis than high-load low-volume exercise. The above reports suggest that the proper implementation of resistance exercise may counteract age-related muscle loss.

Stimulation of muscle protein synthesis by resistance exercise

It was reported that the rate of muscle protein synthesis was inhibited during an acute bout of resistance exercise in humans. However, muscle protein synthesis rapidly resumed during post-exercise recovery. An increase in muscle protein synthesis occurs within the first hour after exercise and can continue for 24 to 48 h. Although older individuals do not seem to have the same magnitude of anabolic response as younger individuals to resistance exercise, older adults still have the capacity to increase muscle protein synthesis in response to resistance exercise. The magnitude of the acute response of muscle protein synthesis to resistance exercise is dependent upon both intensity and workload. Burd et al. reported that low-load (30% of repetition maximum) high-volume resistance exercise is more effective for muscle protein synthesis than high-load low-volume exercise. The above reports suggest that the proper implementation of resistance exercise may counteract age-related muscle loss.

Intracellular pathways for muscle protein synthesis in response to resistance exercise

Muscle protein synthesis increases in response to an acute bout of resistance exercise, and the amount of increase is associated with the activation of mTORC1 signaling in human skeletal muscle (for a review, see 24). The requirement of mTORC1 signaling for exercise-induced activation of muscle protein synthesis was clearly demonstrated in a study that used a specific mTOR inhibitor, rapamycin. Bodine et al. reported that the Akt/mTOR pathway was activated during muscle hypertrophy and down-regulated during muscle atrophy. Furthermore, rapamycin inhibited hypertrophy in all models tested, without causing atrophy in the control muscles. Resistance exercise can also activate the mitogen-activated protein kinase (MAPK) signaling pathway in human skeletal muscle. Williamson et al. demonstrated that resistance exercise increased the phosphorylation of ERK1/2 and mitogen-activated protein kinase-interacting kinase 1 (MNK1) in skeletal muscle of young and old humans. More recently, Miyazaki et al. reported that mTORC1 activation at the early phase of mechanical overload in skeletal muscle occurs independently of the PI3K/Akt pathway. They provided evidence that a MEK/ERK-dependent pathway may contribute to mTORC1 activation through the phosphorylation of TSC2.

In contrast, 5′-AMP-activated protein kinase (AMPK), which is a sensor for the ratio of AMP/ATP, appears to be a negative regulator of protein synthesis. Dreyer et al. reported that muscle protein synthesis decreased and AMPK activity increased during a bout of resistance exercise in human subjects. Other candidate negative regulators of mTORC1 are the mammalian orthologs known as REDD1 and REDD2 (regulated in development and DNA damage responses 1 and 2). It was found that REDD1 inhibits mTOR kinase activity in muscle cells. The role of REDD1/2 in the regulation of mTORC1 activity and protein synthesis in human muscle was also described.

Activation of mTOR pathway by mechanical stimuli

Mechanical stimuli play an important role in the regulation of skeletal muscle mass in response to resistance exercise. Mechanical activation of the mTORC1 pathway was clearly shown by Hornburger et al. These reports demonstrated that mechanical stimuli activate mTORC1 signaling through a PI3K/Akt-independent mechanism. It was shown that the synthesis of phosphatidic acid (PA) by phospholipase D (PLD) is necessary for mechanical activation of mTORC1 signaling. PA has been reported to directly activate mTORC1 signaling by binding to mTORC1 on its FKBP12-rapamycin binding (FRB) domain. The rapamycin-FKBP12 complex can inhibit mTORC1 by binding to the FRB domain. Therefore, mechanical stimuli enhanced PA binding to mTORC1 by mechanical stimuli also activates mTOR and reduces the inhibitory effects of rapamycin. The authors reported that mechanical stretch-induced activation of protein translation initiation and elongation was attenuated by the pretreatment of C2C12 myoblasts with a broad-range tyrosine kinase inhibitor, genistein. This result suggests that stretch-induced activation of protein translation initiation requires tyrosine phosphorylation. More recently, we found that phospholipase C (PLC) and focal adhesion kinase (FAK) might be involved in stretch-induced activation of protein translation. Phosphorylation of PLCγ1 (Y783) and FAK (Y397) was increased by mechanical stretch, and the specific inhibi-
tor of PLC and FAK, respectively, blocked the stretch-induced activation of p70S6K and eEF2 in C2C12 myoblasts (unpublished observation). It was reported that PLCγ1 regulates mTOR/p70S6K activation in leukemic cells, and that suppression of PLCγ1 led to inhibition of cell proliferation and enhanced apoptosis. Another study found that FAK mediates the mechanical stretch-induced activation of cardiac fibroblasts. These findings suggest that PLC and FAK may have important functions in mTORC1 signaling.

The PLC family of cellular proteins plays a significant role in the regulation of various intracellular signaling mechanisms. In addition, phosphatidyl inositol-4,5-bisphosphate (PIP$_2$), a PLC substrate, has signaling capacity by itself and can, by direct interaction, affect the activity and subcellular localization of PLD. Since stimulation of PLC isoforms causes PLD activation, it was assumed that PLD activation might be secondary to the PLC-initiated increase in cytosolic Ca$^{2+}$ and activation of protein kinase C (PKC) isoforms (for review, see 42). In fact, PKC inhibitors and down-regulation of diacyl glycerol kinase (DAG)-dependent PKC reduce receptor-induced PLD responses. Thus, the authors propose that the PLC-PKC-PLD pathway is involved in the mechanical activation of mTORC1. However, further studies are needed to elucidate the molecular mechanisms of the mechanical activation of mTORC1 via lipid second messengers that are produced by PLC and PLD.

**Stimulation of muscle protein synthesis by amino acids**

There are numerous studies demonstrating that the increased availability of amino acids stimulates muscle protein synthesis in humans. This stimulatory effect of amino acids on protein synthesis is primarily due to essential amino acids. Among the essential amino acids, leucine appears to be the most effective for stimulation of skeletal muscle protein synthesis in humans and rodents. However, the addition of leucine to a high-quality essential amino acid solution did not further increase human muscle protein synthesis, although a higher arterial concentration of leucine was observed. This work suggests that intracellular leucine availability may be a regulator of muscle protein synthesis.

Leucine-induced stimulation of muscle protein synthesis has been investigated in greater detail in animal studies. The addition of branched-chain amino acids (BCAAs) to an isolated rat diaphragm and to perfused rat skeletal muscle resulted in stimulation of protein synthesis to the same extent as the addition of a complete mixture of amino acids. Enhanced protein synthesis in rat skeletal muscle following an oral administration of leucine, but not isoleucine and valine, has been reported. Because leucine is an insulin secretagogue, it was speculated that the anabolic effect of leucine in vivo is mediated by insulin. Anthony et al. investigated the relationship between the concentration of serum insulin and protein synthesis after oral administration of leucine to food-deprived rats. Their results suggested that the transient increase in serum insulin facilitated a leucine-induced increase in protein synthesis. In another study, leucine-induced stimulation of protein synthesis was demonstrated in rats with experimentally induced diabetes, suggesting that insulin may play a permissive role in leucine-induced protein synthesis.

**Intracellular pathways for muscle protein synthesis by leucine**

Leucine, as well as insulin and growth factors, stimulates mTOR kinase activity, which then controls the phosphorylation of translation initiation factors. When mTORC1 is activated by leucine, at least two downstream molecules for translation initiation are stimulated. First, mTORC1 regulates the availability of eIF4E via the phosphorylation of 4E-BP1. In addition, mTORC1 phosphorylates p70S6K which, in turn, phosphorylates the S6 ribosomal protein and allows the up-regulation of a subclass of mRNA encoding the translational apparatus. Another potentially important signaling protein in the control of translation is eEF2, which mediates the translocation step of elongation. Activation of eEF2 by dephosphorylation, after amino acid ingestion, was demonstrated in human muscle.

The mechanism responsible for leucine-induced mTORC1 signaling has not yet been clearly defined. However, it is likely that two proteins which are directly associated with kinase, raptor and Rheb, are involved in this process, as reviewed by Kimball and Jefferson. A cell-culture study indicated that under leucine-rich conditions, the mTOR-raptor association is destabilized, resulting in the activation of mTORC1. Over-expression of Rheb results in increased phosphorylation of both 4E-BP1 and p70S6K. The activity of Rheb is controlled, in part, by TSC. Co-expression of TSC1 and TSC2 results in decreased mTOR function, suggesting that the TSC1-TSC2 complex regulates mTOR signaling by inhibiting Rheb. Deprivation of all amino acids, or just leucine, results in the dissociation of Rheb to mTOR. Thus, the results of these in vitro studies suggest that amino acid signaling through mTORC1 requires both Rheb and the TSC1-TSC2 complex.

It was demonstrated that a class III PI3K, hVps34, human vacuolar protein sorting-34, could mediate the leucine-induced activation of mTORC1. The activity of hVps34 is inhibited under instances of amino acid starvation, whereas re-addition of amino acids increases its activity. Another protein that has been implicated as a mediator to mTORC1 is Rag (Ras-related GTPase). The Rag protein was shown to interact with the mTORC1 complex via association with raptor. Overexpression of the active form of Rag activates p70S6K, and mTORC1...
pathway activity becomes insensitive to amino acid deprivation\(^68\). More recently, a novel role for PLD1 in an amino acid-sensing pathway was reported\(^69,70\). It was found that PLD1 is indispensable for amino acid activation of mTORC1, and that the activation of PLD1 by amino acids requires hVps34. The researchers concluded that the hVps34-PLD1 pathway acted independently of, and parallel with, the Rag pathway in regulating amino acid activation of mTORC1\(^69\).

**Relationship between leucine metabolism and muscle protein synthesis**

The stimulatory effect of leucine on protein synthesis may be self-limiting, because leucine promotes its own disposal by activating the branched-chain \(\alpha\)-keto acid dehydrogenase (BCKDH) complex, which is a late-limiting enzyme in the catabolic pathway of the BCAAs. The activity of the BCKDH complex is controlled by covalent modification, in which phosphorylation of the E1 subunit by BCKDH kinase inactivates the complex\(^71\), and dephosphorylation of the E1 subunit by a specific phosphatase reactivates the complex\(^72\). We investigated the relationship between the activity of the BCKDH complex and leucine-mediated activation of protein translation in C2C12 myoblasts\(^73\). As shown in Fig. 2, phosphorylation of p70S6K by leucine was higher in BCKDH E2 subunit knock-down cells (inactivation of the complex), whereas the effect of leucine was diminished in BCKDH kinase knock-down cells (activation of the complex). Insulin-induced phosphorylation of p70S6K was not affected whether BCKDH E2 subunit knock-down cells or kinase knock-down cells were used. In addition, suppression of BCKDH kinase for 48 h resulted in decreased cell proliferation. These results suggest that the activation of translational regulators by leucine was partly regulated by the activity of the BCKDH complex.

Experiments using BCKDH kinase knock-out mice\(^74\) suggested that loss of control over the BCKDH complex inhibits growth, presumably by limiting the availability of BCAAs for protein synthesis and also by decreasing leucine availability to stimulate protein translation. In addition, pharmacological activation of the BCKDH complex by clofibric acid resulted in the suppression of leucine-induced activation of translation initiation processes\(^75\). These findings support the notion that BCAA catabolism, which is mediated by the BCKDH complex, has a significant impact on leucine-induced protein synthesis.

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**Fig. 2** Effect of siRNA transfection on leucine and insulin-induced p70S6K phosphorylation. Leucine (Leu) and insulin (Ins) -induced p70S6K phosphorylation in control-siRNA, E2-siRNA (A) and kinase-siRNA (B) transfected cells were determined by immunoblotting. 48 h posttransfection, cells were washed twice with phosphate-buffered saline (PBS). Then serum-free DMEM/F12 lacking leucine was added to the plates, and the cells were returned to the incubator for 60 min. At that time, the cells were treated with PBS, leucine (2 mM) or insulin (100 nM) for 30 min, and then cells were harvested. Total cell lysates were resolved by 10% SDS-PAGE, transferred onto PVDF membranes and immunoblotted with anti-phospho p70S6K antibody. Photographs of representative Western blots of phospho-p70S6K in control-siRNA, E2-siRNA (A) and kinase-siRNA (B) transfected cells are shown on top. Data are expressed as a percentage of the PBS treated group in control-siRNA transfected cells (means ± SD, n=4). *Significantly different from the PBS group in the same siRNA transfected cells (P < 0.05). #Significantly different from the control-siRNA group with the same treatment (P < 0.05). Data are from Nakai et al.\(^73\).
Additive effect of resistance exercise and nutrition on muscle protein synthesis

There is clear agreement that resistance exercise and amino acid ingestion independently stimulate muscle protein synthesis. It has been reported that amino acid ingestion after resistance exercise results in greater increases in human muscle protein synthesis rates than when amino acids are ingested at rest or when resistance exercise is performed in a fasted state. However, Fujita et al. reported that the ingestion of nutrients before resistance exercise did not increase the rate of muscle protein synthesis compared to exercise alone. The ingestion of amino acids before exercise only prevented an exercise-induced decrease in muscle protein synthesis. Thus, when amino acid ingestion within 24 h after resistance exercise78). A study by West et al. showed that a single 25 g ingested bolus of whey protein enhanced resistance exercise-induced muscle protein synthesis to a greater extent than frequent small-pulse feeding of the identical amount of protein (2.5 g × 10 times over a 3 h period). They also demonstrated that the phosphorylation of p70S6K increased to a greater extent at 1 h post-exercise after bolus ingestion compared to after pulse ingestion. These results suggest that a rapid rise in extracellular amino acid availability during the post-exercise period is effective for the stimulation of muscle protein synthesis.

It was also shown that muscles can respond to amino acid ingestion within 24 h after resistance exercise. A study by West et al. showed that a single 25 g ingested bolus of whey protein enhanced resistance exercise-induced muscle protein synthesis to a greater extent than frequent small-pulse feeding of the identical amount of protein (2.5 g × 10 times over a 3 h period). They also demonstrated that the phosphorylation of p70S6K increased to a greater extent at 1 h post-exercise after bolus ingestion compared to after pulse ingestion. These results suggest that a rapid rise in extracellular amino acid availability during the post-exercise period is effective for the stimulation of muscle protein synthesis after resistance exercise.

Conclusions

It is clear that resistance exercise and ingestion of amino acids stimulate muscle protein synthesis. Current studies of humans and rodents suggest that anabolic signaling, in response to amino acids and resistance exercise, is mediated through mTORC1 signaling in skeletal muscle. Recent progress has also significantly expanded an understanding of the upstream molecules that regulate mTORC1 in response to anabolic stimuli. Resistance exercise and amino acid ingestion independently stimulate muscle protein synthesis. Thus, when amino acid ingestion is combined with resistance exercise, additive effects on protein synthesis can be obtained. Future findings may yield to the development of novel interventions for improving skeletal muscle mass.

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