Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling

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Submitted 21 October 2008; accepted in final form 13 January 2009

Drummond MJ, Dreyer HC, Fry CS, Glynn EL, Rasmussen BB. Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. J Appl Physiol 106:1374–1384, 2009. First published January 15, 2009; doi:10.1152/japplphysiol.91397.2008.—In this review we discuss current findings in the human skeletal muscle literature describing the acute influence of nutrients (leucine-enriched essential amino acids in particular) and resistance exercise on muscle protein synthesis and mammalian target of rapamycin complex 1 (mTORC1) signaling. We show that essential amino acids and an acute bout of resistance exercise independently stimulate human skeletal muscle protein synthesis. It also appears that ingestion of essential amino acids following resistance exercise leads to an even larger increase in the rate of muscle protein synthesis compared with the independent effects of nutrients or muscle contraction. Until recently the cellular mechanisms responsible for controlling the rate of muscle protein synthesis in humans were unknown. In this review, we highlight new studies in humans that have clearly shown the mTORC1 signaling pathway is playing an important regulatory role in controlling muscle protein synthesis in response to nutrients and/or muscle contraction. We propose that essential amino acid ingestion shortly following a bout of resistance exercise is beneficial in promoting skeletal muscle growth and may be useful in counteracting muscle wasting in a variety of conditions such as aging, cancer cachexia, physical inactivity, and perhaps during rehabilitation following trauma or surgery.

HUMAN SKELETAL MUSCLE PROTEIN metabolism has received significant attention over the past few decades because of its relevance to aging, disease processes, and physical inactivity. The importance of skeletal muscle is obvious since it comprises nearly 40% of body weight, constitutes between 50 and 75% of all proteins (73), and is imperative for locomotion. However, it is also important as an amino acid reservoir, for energy consumption and for fuels for other tissues (e.g., brain, immune cells).

Skeletal muscle proteins turnover regularly such that 1–2% of proteins are synthesized and broken down daily (111). The turnover of proteins involves the ongoing processes of protein synthesis and breakdown. A positive net protein balance occurs when proteins accumulate in excess of their removal (e.g., following nutrient ingestion), whereas a negative net protein balance occurs when the breakdown of proteins exceeds that of their synthesis (e.g., fasting). The initial work of Professors Michael J. Rennie and Robert R. Wolfe contributed significantly to the understanding of the muscle protein metabolism field in humans by incorporating stable isotope techniques concurrently with skeletal muscle biopsies in the 1980s and 1990s. Because of these breakthroughs, researchers can now study the dynamics of skeletal muscle protein turnover under various interventions. Although the turnover of skeletal muscle protein is relatively slow compared with other proteins, such as blood (albumin) and gastric intestinal tract proteins, changes in protein turnover can be identified within hours following acute anabolic interventions. Indeed, essential amino acids (particularly leucine) and resistance exercise are powerful stimulators of skeletal muscle protein synthesis in animal and human models (1, 30, 42). Less understood are the cellular and molecular mechanisms regulating protein turnover following nutrition and resistance exercise in humans, although great strides have been made in the last decade. Thus this review highlights the most recent peer-reviewed articles capturing the influence of nutrition and resistance exercise on protein synthesis and cellular signaling in human skeletal muscle.

TRANSLATIONAL CONTROL OF MUSCLE PROTEIN SYNTHESIS

The regulation of skeletal muscle protein turnover is complex but, in general, involves interactions of gene transcription,
transcription and protein breakdown (as well as various pre- and posttranscriptional modifications). This section emphasizes translational control of protein synthesis [particularly translation initiation through the mammalian target of rapamycin (mTOR) pathway].

Translation initiation involves a series of events necessary for ribosomal complex assembly and binding of the target mRNA. A plethora of initiation factors and signaling molecules (through phosphorylation and association steps) regulate the formation of the translational apparatus, thus implicating alternative, but tight, levels of control of the synthesis of new proteins. Interestingly, these regulatory mechanisms can occur within minutes of a particular stimulus (12).

mTOR is a key regulator of translational control. Nutrient, hormonal, and contractile stimuli often (but not always) converge at this protein, suggesting that mTOR is an important modulator of protein synthesis. For example, an initial study conducted by Baar and Esser (3) showed that the mTOR pathway may be associated with long-term increases in muscle mass due to the positive correlation between the acute phosphorylation of the downstream mTOR effector, p70 ribosomal S6 kinase 1 (S6K1), and the increase in muscle mass over 6 wk of electrical stimulation in rodent hindlimb muscles. Interestingly, the association of S6K1 phosphorylation and muscle mass was also demonstrated in human subjects after 12 wk of resistance exercise training (98). To more fully elucidate the role of mTOR, rapamycin, a specific inhibitor of mTOR function, is commonly used to study muscle growth in cell and animal models (2, 61). This was well demonstrated by Bodine and colleagues (11) in which rapamycin was used to block muscle hypertrophy following functional overload in rodents further suggesting an important role for the mTOR pathway in controlling skeletal muscle growth.

The mTOR protein is a very large molecule (289 kDa) with many regulatory domains and is found within two protein complexes: mTORC1 and mTORC2. In addition to mTOR, two known proteins make up the mTORC1 complex [G protein β-subunit-like protein (GβL) and regulatory associated protein of mTOR (raptor)], while the mTORC2 complex is composed of GβL, mSin, and rapamycin-insensitive companion of mTOR (ricktor). The mTORC1 complex is sensitive to rapamycin and plays an important regulatory role during the hypertrophy process of skeletal muscle cells (11). Less is known about the regulation of mTORC2 although it is not thought to be involved in the regulation of translation initiation and elongation (94).

Two well-studied downstream targets of mTORC1 influence translation initiation and elongation: S6K1 and eukaryotic initiation factor 4E binding protein 1 (4E-BP1) (Fig. 1). Additionally, mTORC1 has also been implicated in enhancing ribosomal biogenesis through cMyc (45, 97). S6K1, when activated, can phosphorylate at least nine different targets (92), including ribosomal protein S6 (rpS6) (89) and eukaryotic elongation factor 2 (eEF2) kinase (108). Phosphorylation of rpS6 has been reported to enhance cell size and proliferation (91), although its role has been questioned (91). Reduced phosphorylation of eEF2 due to eEF2 kinase inhibition results in mediation of the ribosome to the mRNA following each amino acid addition, thereby contributing to a growing peptide chain (16). mTORC1 phosphorylation of 4E-BP1 inhibits the binding of eukaryotic initiation factor (eIF) 4E to 4E-BP1, thereby promoting muscle growth.

Figure 1. A simplified schematic representation of the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway driven by muscle contraction, insulin, essential amino acids (leucine), and/or energy. Proteins have been labeled to designate them as a positive (green) or negative (red) regulators of mTORC1 and muscle protein synthesis. AMPK, AMP-activated protein kinase; Akt, protein kinase B; TSC1, tuberous sclerosis complex 1; TSC2, tuberous sclerosis complex 2; REDD1/2, regulated in development and DNA damage responses; Rheb, Ras-homologue enriched in brain; TCTP, translationally controlled tumor protein; PAM, protein associated with Myc; Raptor, regulatory associated protein of mTOR; GβL, G protein β-subunit-like protein; MAP4K3, mitogen activated protein kinase kinase kinase-3; hVps34, human vacuolar protein sorting-34; S6K1, p70 ribosomal S6 kinase 1; 4E-BP1, 4E binding protein 1; eEF2k, eukaryotic elongation factor 2 kinase; eEF2, eukaryotic elongation factor 2; rpS6, ribosomal protein S6; PRAS40, proline-rich Akt substrate-40.
eIF4E to complex with eIF4G, further enhancing formation of the translation initiation complex (109).

There are several proteins that respond to nutrients, hormones, and muscle contraction, which also regulate the function of mTORC1. Rheb (Ras-homologue enriched in brain), the immediate upstream guanosine triphosphate (GTPase), is often targeted in the regulation of mTORC1. For instance, translationally controlled tumor protein (TCTP) has been proposed to catalyze the GDP → GTP reaction, thereby enhancing mTOR activity and cell growth (53). Added to the rapidly growing field of mTORC1 regulation is the E3 ubiquitin ligase, protein associated with Myc (PAM), which has also been postulated to regulate GTP/GDP exchange of Rheb directly thereby activating mTOR (70). Akt (protein kinase B) can directly activate mTORC1 through phosphorylation (76) or indirectly by phosphorylating (and inhibiting) tuberous sclerosis complex 2 (TSC2) (54, 71). mTORC1 is also positively regulated by amino acids, particularly leucine (1, 2). To date, two proteins have been implicated in mediating signals from amino acids to mTORC1: human vacuolar protein sorting-34 (hVps34) and mitogen activated protein kinase kinase kinase kinase-3 (MAP4K3) (17, 38). Interestingly, mTORC1 can also be activated by contraction independent of amino acids, Akt, and growth factors (50, 51). This has been described to occur through phospholipase D 1 and 2 using phosphatidic acid (PA) as a second messenger (52). Unfortunately, few experiments have explored the role of these positive mTORC1 regulators in animal and human skeletal muscle.

On the other hand, REDD (regulated in development and DNA damage responses) 1 and 2 have been identified as negative regulators of mTORC1 (23), possibly through their interaction with TSC2 (29). Another novel negative regulator of mTORC1 is PRAS40 (proline-rich Akt substrate-40). While its function remains elusive, PRAS40 has been shown to bind mTOR via raptor to repress mTORC1 signaling (106, 107). The inhibitory function of PRAS40 is reduced when phosphorylated by Akt or mTORC1 (107). Finally, because synthesizing proteins is an energetically expensive cellular process, it is not surprising to find a specific regulator of mTORC1 function when energy is not sufficient. Indeed, mTORC1 has been shown to be inhibited by AMP-activated protein kinase (AMPK) through enhanced TSC2 activity (55) and, recently, by phosphorylating raptor (47).

Another important signaling pathway involved in regulating translation initiation and elongation is the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway (Fig. 2). Cross talk of the ERK1/2 and mTORC1 signaling pathways has been identified to occur through the ERK1/2 proteins, most likely through direct interaction with TSC2 (67, 88) or indirectly by phosphorylation of p90 ribosomal protein S6 kinase polypeptide 1 (RSK1) (89). ERK1 can also enhance protein synthesis independent of the mTORC1 pathway (40) through MAP kinase-interacting kinase 1 (MNK1) signaling to eIF4E (110).

Much of these data are generated from rodents, Drosophila, and cell lines. Few data, if any, are available on mTORC1 signaling in human skeletal muscle in response to an anabolic stimulus. The following describes current data from human skeletal muscle in which muscle protein synthesis and mTORC1 signaling were assessed following nutrition, resistance exercise, or the two in combination.

**NUTRITIONAL AND CONTRACTILE REGULATION OF HUMAN MUSCLE GENE EXPRESSION**

Traditionally (although now heavily debated), the mTORC1 pathway has been postulated to regulate the transcription of specific genes particularly the 5′ terminal oligopyrimidin (5′ TOP) mRNAs possibly through rpS6. Recent evidence in drosophila has indicated that TOR can increase expression of genes associated with ribosomal biogenesis such as cMyc (45, 97), although the mechanism is not well understood. However, few studies have evaluated the impact of resistance exercise and/or essential amino acids on gene expression responses associated with proteins of the mTORC1 signaling pathway in skeletal muscle. Although the mRNA species is a precursor to the proteins that influence translation initiation and elongation, repeated transient changes in genes may influence specific protein accumulation or removal. For instance, our research group identified a decrease in basal Akt and S6K1 mRNA expression after 10 wk of paraplegia in rodents compared with healthy controls (35). This finding paralleled the decreased protein expression of Akt and S6K1 and a concomitant decrease in protein synthesis (31). In another study, our laboratory evaluated the expression pattern of mRNAs associated with the mTORC1 pathway following a single bout of low-intensity resistance exercise in human skeletal muscle. Although mTOR and S6K1 mRNA expression were unchanged 3 h postexercise, REDD1 mRNA expression, a negative regulator of mTORC1, was significantly decreased (34). The rapid change in REDD1 mRNA expression was not entirely surprising because REDD1 protein has a fast turnover rate (<5 min) (62), but instead suggests that acute changes in the mRNAs associated with the regulation of mTORC1 may indirectly impact the function of this integral protein. In another recent publication, our laboratory found that Rheb mRNA expression, the primary positive regulator of mTORC1, was increased, whereas TSC1 and TSC2 mRNA was decreased in human skeletal muscle within hours of resistance exercise and essential amino acid ingestion (36). Thus it is tempting to speculate that with repeated bouts of exercise and/or supplementation with essential amino acids, transient changes in expression of mTORC1 pathway-related genes may change expression of specific proteins over time.

**CONTRACTILE REGULATION OF HUMAN MUSCLE PROTEIN SYNTHESIS**

Early work demonstrated that exercise produces significant changes in whole body protein metabolism and hinted at the possibility that muscle protein turnover was depressed (84, 85). Our laboratory confirmed that muscle protein synthesis was indeed depressed “during” an acute bout of resistance exercise (30). However, the inhibition of muscle protein synthesis that occurs during muscle contraction is rapidly reversed during postexercise recovery. For example, several human studies in the early 1990s demonstrated that an acute bout of resistance exercise stimulated muscle protein synthesis during the first few hours of recovery (8, 21, 68, 113). From these and later investigations it is now well established that a single bout of resistance exercise, even when performed in the fasted state, increases human skeletal muscle protein synthesis.

Muscle protein breakdown is also increased after resistance exercise (80, 81). A positive correlation has been identified
between the rate of muscle protein synthesis and the rate of muscle protein breakdown 3 h following resistance exercise, suggesting a link between the two processes during the early recovery hours (80). A potential mechanism for this association may be that high-resistance muscle contractions increased the activity of the nutrient sensor hVps34 (69), a protein identified as being an amino acid sensor that activates mTORC1 (17) and an activator of autophagy through its binding partner hVps15 and beclin-1 (4). It has been suggested that an increased S6K1 activity (which correlates with increased hVps34 activity) may concurrently stimulate muscle protein synthesis and protein breakdown in rodents (69). Nonetheless, muscle protein synthesis is stimulated to a greater extent than breakdown, leading others to propose that the higher rate of protein synthesis is a key factor in promoting skeletal muscle hypertrophy due to resistance exercise training (80).

The mechanisms for the increase in human muscle protein synthesis following resistance exercise were relatively unknown a few years ago. Recent work has begun to combine traditional stable isotope infusion studies with analysis of key cell signaling pathways known to be involved in the regulation of translation initiation and protein synthesis following human muscle contraction (30, 37, 44). From these initial studies, it appears that activation of the mTORC1 signaling pathway is playing an important role in stimulating muscle protein synthesis following muscle contraction. For example, a key downstream effector of mTORC1, S6K1, is readily phosphorylated in the first 1–6 h following a bout of resistance exercise concurrently with an increase in muscle protein synthesis (30, 44). Another downstream effector of mTORC1 is 4E-BP1, and our laboratory has consistently shown that 4E-BP1 phosphorylation is reduced “during” resistance exercise and is unchanged during postexercise recovery (30, 32). Thus, in our
laboratory’s hands, it appears that the contraction induced increase in muscle protein synthesis is independent of changes in 4E-BP1 phosphorylation which is in stark contrast to the large increase in 4E-BP1 phosphorylation detected when essential amino acids are ingested (32, 33).

Time course studies for the signaling events discussed above have also been carried out because previous work has shown that the rate of human skeletal muscle protein synthesis remains elevated for up to 24 h in trained individuals (68) and for 48 h (80) and 72 h (74) in untrained individuals. Deldicque and colleagues (27) showed that S6K1 phosphorylation remained elevated at 24 and 72 h after completing 10 sets of 10 repetitions of leg exercise performed at 80% of a one repetition maximum (27). This suggests that sustained phosphorylation of S6K1 may play a role in maintaining synthesis rates of muscle proteins above baseline for similar durations in untrained individuals. At present, no concurrent measurements of human muscle protein synthesis and mTORC1 signaling data exist for time points greater than 6 h postresistance exercise. However, it should be noted that other studies have shown increases in muscle protein synthesis using different modes of contraction such as endurance exercise (19) and dynamic shortening and lengthening exercise (26). It is likely that different modes of contraction may result in a somewhat different cell signaling response.

Resistance exercise in humans has also been shown to alter other components of the mTORC1 signaling pathway including Akt and eukaryotic elongation factor 2 (eEF2). Akt is an upstream regulator of mTORC1 (Fig. 1) which is responsive to both insulin and muscle contraction. Akt phosphorylation is increased at 10 min (24) and at 1 and 3 h postresistance exercise (30, 44). However, changes in Akt phosphorylation have not been shown in all studies (33, 37, 72, 96). Possible explanations for the varying results may include glycosgen status, differences in exercise intensity, antibody specificity, and isoform expression.

Our laboratory has recently shown that translation elongation also appears to be sensitive to muscle contraction since eEF2 phosphorylation is decreased (indicator of enhanced elongation) following resistance exercise (30, 33). Contractile regulation of eEF2 may be mediated by S6K1 phosphorylation (and inactivation) of eEF2 kinase (108). It has also been shown that resistance exercise decreases the phosphorylation of eIF2Be, which likely plays a key regulatory role in stimulating muscle protein synthesis during recovery (44). Although these studies suggest that the mTORC1 pathway is involved in regulating muscle protein synthesis postexercise, it should be acknowledged that other important signaling pathways which are not necessarily a component of the mTORC1 network may also be involved in regulating muscle protein synthesis such as the ERK1/2 pathway (Fig. 2). Our laboratory and others have shown that ERK1/2 and MNK1 are readily phosphorylated during postexercise recovery (33, 112). Our laboratory has recently proposed that maximal stimulation of muscle protein synthesis during postexercise recovery likely requires dual activation of both the mTORC1 and the ERK1/2 signaling pathways; however, much more work needs to be done in this area (33). It should be noted that some studies fail to demonstrate significant increases in the phosphorylation of some components of these signaling pathways, perhaps due to differences in exercise intensity (57), mode of contraction (37), and/or glycogen status (24). In any event, evidence from the data highlighted in this section clearly shows that the increase in muscle protein synthesis following a bout of resistance exercise is associated with an activation of the mTORC1 signaling pathway in humans.

NUTRITIONAL CONTROL OF HUMAN MUSCLE PROTEIN SYNTHESIS

The effect of nutrients on muscle protein turnover was originally measured in humans using stable-isotopic techniques in vivo by Rennie et al. in 1982 (86). Stable isotopic methodologies developed over the past 25 yr as well as advances in measuring intracellular signaling events and gene expression have greatly advanced our knowledge of nutritional regulation of muscle protein turnover. Although earlier studies showed increases in muscle protein synthesis of 30–100% following ingestion of mixed amino acids (6, 86), we now know that this can primarily be attributed to essential amino acids alone (104).

Ingestion of essential amino acids with or without carbohydrate has been shown to increase muscle protein synthesis (42, 104). In humans, increases in protein synthesis caused by the ingestion of essential amino acids in combination with carbohydrate are associated with increased mTORC1 signaling as well (25, 42). In particular, our laboratory previously reported that phosphorylation of Akt, mTOR, 4E-BP1 and S6K1 were increased and eEF2 was decreased in association with an ~100% increase in muscle protein synthesis following ingestion of a leucine-enriched essential amino acid and carbohydrate nutrient solution (42). The branched-chain amino acid leucine has received much attention for its ability to increase muscle protein synthesis and activate mTORC1 without the addition of other essential amino acids (2). The specific cellular mechanism for the unique ability of leucine to independently stimulate muscle protein synthesis remains to be elucidated, although evidence in animal models indicates (see below) it is most likely through enhanced translation initiation (1, 2, 13).

The role of carbohydrate on protein turnover has been less clearly defined. The major consensus is that essential amino acids stimulate protein synthesis but do not affect breakdown, while insulin reduces protein breakdown with little to no significant effect on protein synthesis if blood amino acid concentrations are not maintained (7, 22, 25, 41). A recent study by Chow et al. (22) comparing several surrogate measures of muscle protein synthesis and breakdown with measures of amino-acyl tRNA (a precursor for synthesis) and mass balance of trace amino acid (for breakdown) confirmed these hypotheses (22). Other studies examining the effect of carbohydrate following resistance exercise have also shown a decrease in protein breakdown with either local hyperinsulinemia (10) or ingestion of carbohydrate (15) with no additional effect on protein synthesis. Carbohydrates may also affect AMPK, decreasing its activation and thereby reducing the AMPK inhibition of the mTOR pathway (for a review, see Ref. 49).

New evidence has revealed that essential amino acids may not stimulate the mTORC1 pathway by activating the p85/p110 phosphatidylinositol 3-kinases (PI3K) and downstream targets PDK1 and Akt, traditionally seen in insulin and growth factor signaling (77). Instead, new data from cell culture and Dro sophila experiments demonstrate that essential amino acids can
directly stimulate mTOR via a recently characterized class 3 phosphatidylinositol kinase, hVps34 (17) as well as through the Sterile-20 (Ste20) protein kinase, MAP4K3 (38). A family of small GTPases known as Rag proteins may also be important in promoting the intracellular localization of mTORC1 toward Rheb during amino acid stimulation, although this has only been observed in human embryonic kidney cell cultures to date (93). Currently, mTORC1 is thought to be regulated by at least three known inputs: glucose via insulin signaling stimulation and decreased AMPK activation (20, 55); amino acids via hVps34 (17), MAP4K3 (38) and the Rag GTPases (93); and growth factors through Akt and the TSC1/2 complex (66).

Nutrients can directly or indirectly affect each of these inputs, confirming the importance of nutrition in regulating the mTORC1 pathway.

CONTRACTILE AND NUTRITIONAL REGULATION OF HUMAN MUSCLE PROTEIN SYNTHESIS

Resistance exercise is clearly a strong stimulus for promoting skeletal muscle hypertrophy. A single bout of resistance exercise increases both the rate of muscle protein synthesis and the rate of muscle protein breakdown, and thus the net balance of muscle proteins remains negative in the absence of nutritional intake. Muscle protein accretion can only occur with a positive net balance of proteins. Protein, amino acids, and carbohydrate ingestion following resistance exercise improves net muscle protein balance by stimulating muscle protein synthesis and possibly by also inhibiting muscle protein breakdown. Increasing the availability of amino acids provides substrate for de novo protein synthesis and also leads to an increase in the circulating plasma insulin concentration (39). For example, an intravenous administration of amino acids following a bout of resistance exercise stimulates muscle protein synthesis (9). The oral administration of amino acids has also been studied, as a more practical delivery method for increasing amino acid availability to the muscle, and large amounts of amino acids ingested after a bout of exercise also stimulates muscle protein synthesis (15, 75, 99–101). When smaller amounts of amino acids are ingested, both with and without carbohydrate, similar increases in postresistance exercise muscle protein synthesis are observed (14, 83). The increase in muscle protein synthesis observed with the ingestion of amino acids following exercise can likely be explained by the increase in intracellular availability of amino acids activating mTORC1 (42). Another explanation is that increased muscle protein synthesis is due to a particular amino acid or group of amino acids, such as the branched-chain amino acids. A recent paper suggests that the addition of leucine to protein hydrolysate and carbohydrate led to a greater increase in protein synthesis than protein hydrolysate and carbohydrate without leucine alone following resistance exercise (63). The added leucine may exert its effects through an increase in plasma insulin concentrations and/or through a more pronounced activation of mTORC1 signaling pathway (63).

Following a bout of resistance exercise, the ingestion of carbohydrate in the form of glucose resulted in a decrease in urea excretion, typical of a reduction in muscle protein breakdown (90). The ingestion of carbohydrate following exercise does not stimulate muscle protein synthesis, so net protein balance remains negative (15). The changes in muscle protein metabolism can be attributed to the increase in plasma insulin concentrations, although insulin has only a modest effect on protein synthesis in the absence of amino acids (7).

Our laboratory and others have studied the effects of a combination of amino acids and carbohydrate on postresistance exercise muscle recovery. Not only has it been shown that muscle protein synthesis is stimulated to a larger extent than with nutrients or exercise alone but also that the addition of carbohydrate to amino acids does not have an additive effect when ample amounts of amino acids are ingested (15, 42). Our laboratory recently reported an enhanced activation of muscle protein synthesis and a substantial increase in phosphorylation of Akt, mTOR, S6K1, and 4E-BP1 with ingestion of nutrients (leucine-enriched essential amino acids + carbohydrate) 1 h following a bout of resistance exercise, indicating improved translation initiation (32). Translation elongation also appeared to be enhanced, as seen by a decrease in the phosphorylation of eEF2 (32). One previous study found that ingestion of a small amount of essential amino acids and carbohydrate immediately before a bout of resistance exercise resulted in a higher rate of estimated muscle protein synthesis than when the nutrients were ingested immediately postexercise (100). However, when using direct measures of muscle protein synthesis (i.e., fractional synthetic rate), the ingestion of nutrients following exercise has been shown to yield the greatest increase in muscle protein synthesis (32). This is similar to resistance exercise (30) and nutrient ingestion (42) individually or when nutrients are provided before resistance exercise (43) (Fig. 3). This potent anabolic muscle protein synthesis response is supported with enhanced mTORC1 signaling, including a robust phosphorylation of S6K1 following the intervention (32). The addition of carbohydrate to an amino acid nutritional supplement following resistance exercise may improve net protein synthesis and perhaps reduce muscle protein breakdown.

**Muscle Protein Synthesis**

**Fig. 3.** Effect of resistance exercise and the timing of nutrient (leucine-enriched essential amino acids + carbohydrates) ingestion on muscle protein synthesis in young human subjects. Data are presented as percent change in fractional synthetic rate (FSR) from baseline. Resistance Exercise, percent increase in FSR for 2-h period of postexercise recovery (n = 11; 7 men and 4 women) (Ref. 30); Nutrient Ingestion before Resistance Exercise, percent increase in FSR for 2 h period of postexercise recovery (nutrients ingested 1 h before exercise) [n = 11, 6 men (Ref. 43) and new data from 5 female subjects]; Nutrient Ingestion, percent increase in FSR 1 h following nutrient ingestion (n = 11; 6 men and 5 women) (Ref. 42); Nutrient Ingestion after Resistance Exercise, percent increase in FSR 1 h following nutrient ingestion when nutrients were ingested 1 h after resistance exercise (n = 6 men) (Ref. 30). *Significantly greater than baseline, P < 0.05. **Significantly greater than nutrient ingestion before resistance exercise, P < 0.05.

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protein balance through a decrease in muscle protein breakdown and/or by increasing insulin levels to support muscle protein synthesis (15).

Our laboratory published a study identifying the effects of ingesting an essential amino acid and carbohydrate solution before a bout of heavy resistance exercise on muscle protein synthesis (43). Ingestion of nutrients before exercise did not improve the anabolic muscle protein synthesis response compared with resistance exercise alone (30, 43). The ingestion of amino acids before resistance exercise did prevent the exercise-induced decrease in muscle protein synthesis, as identified previously when resistance exercise is performed in the fasted state (30), but did not improve the muscle protein synthesis response following the bout of exercise compared with resistance exercise in the fasted state. However, a recent paper has shown that when protein is ingested during a 2-h workout that includes resistance exercise, muscle protein synthesis was increased (5), although the increase was much less than seen when essential amino acids are ingested postexercise (32). These recent findings suggest that the increased availability of amino acids and elevated plasma insulin levels during postexercise recovery enhance muscle protein synthesis likely via activation of the Akt/mTORC1 signaling pathway.

AGING, NUTRITION, AND RESISTANCE EXERCISE

Numerous studies have examined the cellular and molecular mechanisms associated with the decline in muscle mass that accompanies aging. Because basal rates of muscle protein synthesis are similar between age groups (103), older humans may be impaired in their ability to properly respond to an anabolic stimulus (e.g., resistance exercise and/or essential amino acids) compared with the young. Volpi and colleagues (102) identified that a 3-h amino acid infusion stimulated muscle protein synthesis to a similar extent in young and older subjects. However, in response to ingestion of essential amino acids, older subjects are less sensitive to smaller doses of essential amino acids (7 vs. 15 g) compared with the young (58, 78). Cuthbertson et al. (25) and Guillette et al. (46) have shown that the reduced anabolic response to nutrients in older adults may be associated with dysregulated mTORC1 signaling. However, it appears that adding additional leucine to a nutritional supplement in older adults may be necessary to optimally stimulate muscle protein synthesis to a similar rate as in the young (59). Interestingly, Rieu et al. (87) supplemented meals with leucine, which restored the anabolic effects of feeding on muscle protein synthesis in older adults. Therefore, it may be important for older individuals to eat a sufficient amount of protein with each meal to ensure sufficient leucine availability for maximizing muscle protein synthesis during feeding (79). Although it should be kept in mind that a high-protein diet in older adults may present some physiological concerns. For example, in one study (105), older subjects demonstrated a tendency for a reduced glomerular filtration rate compared with healthy young subjects, who significantly increased glomerular filtration rate following the high protein diet (3 g protein/kg fat-free mass). This may result in a detrimental impact on renal function if continued over time. Together, ingesting a sufficient amount of essential amino acids can stimulate muscle protein synthesis in older adults to a similar extent as in the young (102).

As mentioned previously, resistance exercise is an excellent way to stimulate muscle protein synthesis. However, earlier work indicated that muscle protein synthesis was unchanged in older adults 3 h following a single bout of heavy resistance exercise compared with the increase seen in the younger subjects (95). Additionally, a study by Kumar et al. (65) showed that older adults have a blunted muscle protein synthesis response compared with young adults following resistance exercise (60–90% 1 repetition maximum). These findings are novel and support the idea of an impaired ability in older adults to respond to a protein anabolic stimulus, with the end result being an acute dysregulation of muscle protein synthesis. The signaling pathways associated with acute dysregulation in muscle protein synthesis following resistance exercise in older adults are not yet clear. Several articles have indicated an altered anabolic response at the gene and protein level during the early recovery hours following resistance exercise in older human skeletal muscle. The impairment covers a vast array of molecules from anabolic and cell cycle remodeling and inflammatory genes and proteins (18, 28, 33, 36, 56, 60, 112). In previous work, our laboratory suggested that older men were not able to generate the same level of muscular tension as the young during a bout of heavy resistance exercise (33). In addition to the suggested age-related differences in fiber type, muscle mass and postexercise resistance lactate responses, we attributed this claim to a dysregulation in translation initiation-associated cellular signaling. Following resistance exercise, older subjects had a tendency for lower S6K1 phosphorylation but, more interesting, a blunted ERK1/2 and MNK1 signaling compared with the young when exercising at the same relative exercise intensity (33). Although mTORC1 cellular signaling responses were somewhat similar between age groups in our laboratory’s study (33), we propose, as mentioned previously, that ERK1/2 and mTORC1 pathways must be dually stimulated to achieve maximal activation of muscle protein synthesis during the early recovery hours following resistance exercise. In support of this hypothesis, IGF-I induced muscle hypertrophy was blunted (reduced S6K1 phosphorylation) following inhibition of the ERK1/2 pathway in rodent skeletal muscle (48). Thus it is tempting to speculate that a specific site on S6K1 is targeted (directly or indirectly) by ERK1/2 and that a lack of ERK1/2 activity would prevent the activation of all S6K1 phosphorylation sites that are required for full activity of this enzyme (82). However, this may not be the case when essential amino acids are ingested. According to Karlsson et al. (57), the branch-chained amino acids activate translation initiation that is dependent on mTORC1, but not ERK1/2 signaling in humans. Together, these data suggest that an impaired ERK1/2 signaling pathway may be associated with the blunted muscle protein synthesis response in older subjects following an acute bout of resistance exercise. Further work is needed to evaluate whether additional cellular pathways contribute to the acute impaired muscle protein synthesis response in aging human skeletal muscle.

Over the last couple of years, the ingestion of essential amino acids or protein following resistance exercise has been evaluated in older humans. In these studies, muscle protein synthesis was similar in young and older men 6 h following resistance exercise (33, 64). Interestingly, muscle protein synthesis was delayed at 3 h postexercise but was restored a few
hours later (33). Although the muscle protein synthesis response was similar over the 6 h postresistance exercise period in both age groups, we are unsure whether this muscle protein synthesis response remains the same or diverges over a longer time course (e.g., 24 h postresistance exercise). Further research is required to evaluate the effectiveness of essential amino acid supplementation following repeated bouts of resistance exercise to determine whether the muscle protein synthesis response continues to be similar between young and older subjects.

**SUMMARY AND CONCLUSIONS**

In summary, human skeletal muscle protein synthesis is increased following the ingestion of essential amino acids + carbohydrates and following an acute bout of resistance exercise. However, muscle protein synthesis is increased to a greater extent when the nutrients are ingested following resistance exercise. Our data, as well as others, indicate that the mTORC1 signaling pathway and alterations in the expression of key genes associated with the regulation of protein turnover may contribute to the acute increase in muscle protein synthesis response following resistance exercise and nutrition. Furthermore, muscle protein synthesis is impaired in the old following a single bout of resistance exercise in conjunction with altered anabolic gene and cell signaling responses. Providing nutrition following resistance exercise in older adults appears to increase muscle protein synthesis to levels similar to the young but in a delayed manner. We conclude that essential amino acids and/or resistance exercise stimulate human muscle protein synthesis in association with enhanced mTORC1 signaling. By better understanding the cellular mechanisms regulating muscle protein synthesis, we are now able to scientifically design exercise and nutritional strategies to include ingestion of leucine-enriched essential amino acids following resistance exercise to maximally promote muscle protein synthesis and growth. These strategies may have important clinical application in patients with muscle wasting conditions such as aging, cancer, physical inactivity/bed rest, and they may be useful in improving muscle rehabilitation following trauma and/or surgery.

**GRANTS**

This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Disease Grant R01 AR-049877. Additional support came from Grant P30 AG-024832 [National Institutes of Health (NIH)/National Institute on Aging] and M01 RR-00073 from the General Clinical Research Branch, National Center for Research Resources (NIH).

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