Review


HIGHLIGHTED TOPIC  |  Regulation of Protein Metabolism in Exercise and Recovery

Regulatory mechanisms of skeletal muscle protein turnover during exercise

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DURING EXERCISE WHOLE BODY energy turnover increases greatly and this is mainly due to higher ATP turnover by working skeletal muscles. In the mid-1800s the German physiologist von Liebig surmised (100) that the primary fuel for energy turnover in working skeletal muscle was intramuscular protein and that large amounts of meat should be eaten to refill this store. Although the habitual dogma of eating protein-rich foods after exercise may still be popular, it is now clear that von Liebig was incorrect because protein catabolism contributes very little to energy turnover in working muscle, and carbohydrates and lipids are the main substrates utilized in working rat (42) and human (55) muscle, the selection of which depends on exercise intensity and duration as well as the preexercise diet (1, 17, 40, 83, 99). However, it should be noted that amino acids still contribute to oxidative metabolism in skeletal muscle during exercise depending on exercise duration and diet as well as training status, and this is explained in detail in several other comprehensive reviews (37, 78, 101).

Even though protein may not contribute much to energy turnover, there are changes in protein metabolism in working muscle. This review will not focus on the regulation of mechanisms of amino acid catabolism or uptake with exercise, and the reader is referred to several other detailed reviews (78, 80, 81, 101) on these topics. Instead, the aim of this review is to summarize the studies that have examined protein turnover in working muscle during exercise and describe the possible molecular mechanisms involved therein. It is believed that the understanding of these mechanisms behind changes in muscle protein turnover during such a stress as exercise may lead to development of new strategies and treatments for individuals with muscle-wasting conditions such as malnutrition, cancer cachexia, sepsis, and immobility (78, 79).

SKELETAL MUSCLE PROTEIN TURNOVER DURING EXERCISE

Shown in Fig. 1 is a general schema of protein and amino acid metabolism in skeletal muscle. Skeletal muscle can take up or excrete amino acids from the blood via specific amino acid transport proteins, most of which are Na⁺ dependent (78, 80). The free amino acid pool is in a balance between cellular uptake/release and intracellular catabolism and anabolism. Protein turnover is defined as the balance of the synthesis and degradation of myocellular proteins from and to the intracellular free amino acid pool, respectively (Fig. 1). When compared with other tissues such as the liver and gut, skeletal muscle protein turnover is relatively slow, and it only varies

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slightly during fasting and feeding, with net breakdown and net synthesis occurring during these times, respectively (101). Nonetheless, it has been clear for some time (35) that skeletal muscle protein is in a continuous state of flux and can increase or decrease depending on diverse physiological stimuli.

In contrast to the vast amount of studies on carbohydrate and lipid metabolism, there are relatively few studies on protein turnover during exercise. Also, most studies that have examined skeletal muscle protein turnover and exercise have investigated the postexercise period when there is typically a positive net protein balance (75, 78, 81).

**REGULATION OF SKELETAL MUSCLE PROTEIN SYNTHESIS DURING EXERCISE**

In rats, tracer studies show that in vivo skeletal muscle protein synthesis decreases by about 30% during contractile activity in the conscious, unrestrained state (25) as well as during electrical stimulation in situ (Ref. 14; Fig. 2A) and ex vivo (62, 70). Furthermore, recent ex vivo studies (62) demonstrated that contraction inhibited basal protein synthesis by about 70% and was able to fully override the anabolic effect of insulin. No definitive evidence had shown that muscle protein synthesis was blunted during exercise in humans (81, 101) until the recent report by Dreyer et al. (28) that showed that protein synthesis rates in vastus lateralis muscle were depressed by about 30% during resistance exercise-type work (Fig. 2B). Although no study has categorically shown that endurance-type exercise blunts skeletal muscle protein synthesis in humans, an earlier study (16) did demonstrate a slight reduction, albeit nonsignificant, in quadriceps muscle protein synthesis during 4 h of treadmill walking. Obviously, further studies are warranted to clarify this issue. Also, it is clear that nutritional status is important because preexercise feeding of carbohydrate and amino acids can blunt the decrease in muscle protein synthesis during resistance exercise observed in the fasted state (33) and feeding before and during resistance exercise actually increases skeletal muscle protein synthesis compared with the basal state (6). By nature, resistance exercise is intermittent, and whether intake of macronutrients influences the suppression of protein synthesis in working muscle during continuous, endurance-type exercise remains to be examined. In any case, it is clear that the inhibition of skeletal muscle protein synthesis during exercise is incomplete, and this is exemplified by the evidence that skeletal muscle can actually synthesize proteins such as interleukin-6 (58a, 93) and heat shock protein 72 (32) during exercise.

So, what could be the mechanism(s) behind the depressed protein synthesis during contractile activity? Some studies suggest that changes in hormonal levels of insulin, epinephrine, and glucocorticoids during exercise may be contributing factors (35, 106). However, because there can be a decrease in muscle protein synthesis rate during contractions of isolated (62, 70) or perfused (14) rat skeletal muscle, this decrease is likely to result from factors arising within the contracting muscle.
skeletal muscle itself and not humoral factors. Indeed, if anything, stimulation of β-adrenergic signaling probably stimulates skeletal muscle protein synthesis (58).

A simple explanation may be that the supply of messenger RNA or free amino acids may limit the supply of template or building blocks for the protein synthesis machinery. However, neither the total amount of mRNA (73) or free amino acids (14, 23, 38, 41) is altered in contracting skeletal muscle during short-term exercise or contractile activity. Thus it is likely to be that muscle contractions alter the mRNA translational machinery. Indeed, because the blunting of skeletal muscle protein synthesis with contractions was relatively fast, Bylund-Fellenius et al. (14) concluded that the control of this must be via a rapid mechanism such as inhibition of peptide chain translation initiation or elongation and not via the number and distribution of ribosomes or the amount of translatable mRNA, which were supposed to be control points in long-term adaptive responses. In line with this, Dohn et al. (24) demonstrated that there was lower amino acid incorporation in vitro by isolated polyribosomes from exercised vs. sedentary rats.

Translation is conventionally divided into three phases: initiation, elongation, and termination controlled by proteins called eukaryotic initiation, elongation, and release factors, respectively (74). To gauge whether translation initiation or elongation steps are the major rate-limiting steps behind changes in protein synthesis, one can examine the ribosomal distribution between aggregated polysomes and disaggregated monosomes. In theory, if there were a selective brake on initiation vs. elongation, then there would be a disaggregation of polysomes and accumulation of monosomes in the cell (48). Two studies from Jefferson’s laboratory (14, 106) have examined the effects of exercise/contraction on ribosomal aggregation in skeletal muscle samples by spectrophotometric analysis of density-gradient centrifugation fractions. In the earlier study of rats (14), no difference in the abundance of 40S or 60S monosomes were observed despite detecting lower skeletal muscle protein synthesis during contractions, and it was concluded that a selective impairment on peptide chain initiation did not occur and that the blunting of translation was probably a combination of impaired initiation and elongation. In the more recent study (106), a shift in ribosomes from polysomes to monosomes (40S, 60S, and 80S) was shown to occur in muscles from running compared with resting mice, and the authors concluded that initiation was inhibited relative to elongation. However, discrepancies between these studies were not discussed in the later (106) paper. In any case, further studies are required to clarify this issue.

REGULATION OF mRNA TRANSLATION INITIATION

Translation initiation is a major site of control of peptide synthesis (74). An initial step involves recruitment of the initiator methionyl-tRNA to the ribosomal subunit, which is catalyzed by GTP-bound eukaryotic initiation factor (eIF) 2α. eIF2α-GDP is reactivated by eIF2B, and eIF2α activity can be blocked by competitive binding of phosphorylated eIF2α to eIF2B (74). Because phosphorylation of eIF2α can occur as a response to cellular stress (84), it could be that this may be a regulatory mechanism for the blunting of skeletal muscle protein synthesis with exercise. However, studies show no effect of acute resistance exercise (10) or ex vivo contraction (62) on eIF2α phosphorylation in skeletal muscle of rats. Another important step of initiation is 40S ribosomal subunit binding to the mRNA which is catalysed by a complex of eIF4A-eIF4E-eIF4G (74). A major regulator of the activity of this complex is eIF4E binding proteins (4E-BP), which bind eIF4E when dephosphorylated thereby preventing this initiation complex formation. During feeding of rats, 4E-BP1 phosphorylation increases which enables greater formation of the eIF4A-eIF4E-eIF4G complex in skeletal muscle (9). In contrast, during exercise/contraction, several studies have now shown that 4E-BP1 phosphorylation decreases in skeletal muscle of humans (20, 28, 59, 85) and rodents (3, 106) and thus may be a potential mechanism behind the blunting of protein synthesis. Indeed, cell culture studies consistently showed lower 4E-BP1 phosphorylation and protein synthesis in response to a variety of different stressors (72). However, our laboratory has recently shown dissociation between the magnitudes of the decrease in protein synthesis and 4E-BP1 phosphorylation in rat skeletal muscle during ex vivo contractions (84a). Clearly, further work is required to determine the importance, if any, of 4E-BP1 dephosphorylation during exercise.

Lastly, one study (106) has shown that eIF4E phosphorylation is higher in skeletal muscle of running mice and suggested that this would affect translation of certain mRNAs but probably not global protein synthesis.

REGULATION OF mRNA TRANSLATION ELONGATION

Translation elongation is also an important control step of peptide synthesis (74). In particular, in situ treatment of skeletal muscles with cycloheximide, a chemical that blocks eukaryotic mRNA elongation (39), results in a near complete blunting of skeletal muscle protein synthesis (48), illustrating that blockade of elongation is a potential means by which exercise may blunt protein synthesis in working muscle. In mammalian cells, peptide chain elongation requires two eukaryotic elongation factors (eEF), namely eEF1 and eEF2 (74). eEF1 is actually a complex of eEF1A, which binds GTP and recruits aminoacyl-tRNAs to the ribosome, and eEF1B, which reactivates eEF1A-GDP and is regulated by reversible phosphorylation (74). Because there is little known about skeletal muscle eEF1 complex, it will not be discussed further. eEF2 mediates the translocation of the ribosome relative to the mRNA after addition of each amino acid to the nascent chain (13). The activity of eEF2 is regulated by reversible phosphorylation within the GTP-binding domain at Thr56 (76). This phosphorylation inhibits eEF2 activity by preventing eEF2 binding to the ribosome (15), thereby impairing elongation rate (76, 77, 89). Some studies (Fig. 3) show that eEF2 phosphorylation is higher in working skeletal muscle during cycling exercise in humans (85, 86) as well as ex vivo (3, 62). However, this finding is not consistent, with other studies showing that skeletal muscle eEF2 phosphorylation was unchanged in humans performing endurance (59) or resistance (20, 28) exercise. The latter may be explained by the findings that eEF2 phosphorylation was increased only during low-frequency endurance exercise-type, but not high-frequency, resistance exercise-type, stimulation of rat skeletal muscles (3). In any case, our laboratory consistently observes increases in skeletal muscle eEF2 phosphorylation in studies from humans and rodents with exercise/contraction provided that the muscle
Two principal signaling events: translation factors during exercise/contraction originate from size that the intracellular signals leading to inhibition of to the regulation of muscle glucose transport (87), we hypothesis in working skeletal muscle are illustrated in Fig. 4. Similar SIGNALING TO TRANSLATION FACTORS further light on this issue. of either eEF2 or eEF2 kinase in skeletal muscle may shed transgenic mice that express mutants or knockout or silencing contractions. Further studies using muscle cell cultures or sample is handled carefully and frozen rapidly after excision (for example see Fig. 3). To date, no study has reported a functional consequence of skeletal muscle eEF2 phosphorylation during exercise, however, our laboratory (84a) has shown that treatment of isolated rat skeletal muscles with eEF2 kinase inhibitors fully blocks eEF2 phosphorylation and partially (−30−40%) blunts the inhibition of protein synthesis with contractions. Further studies using muscle cell cultures or transgenic mice that express mutants or knockout or silencing of either eEF2 or eEF2 kinase in skeletal muscle may shed further light on this issue.

SIGNALING TO TRANSLATION FACTORS

The signaling mechanisms behind the fall in protein synthesis in working skeletal muscle are illustrated in Fig. 4. Similar to the regulation of muscle glucose transport (87), we hypothesize that the intracellular signals leading to inhibition of translation factors during exercise/contraction originate from two principal signaling events: 1) the repeated spikes in free Ca$^{2+}$ concentration, and 2) the chemical changes that occur as a result catabolism with contraction. Studies of cells (57, 68, 108) and isolated rat muscles (47, 58) have also shown that treatment with agents that raise intracellular Ca$^{2+}$ blunts protein synthesis. However, studies of the specific role of Ca$^{2+}$ signaling on metabolism are often complicated by the resulting alterations in energy turnover (49, 57). Thus, to investigate this hypothesis further, we have performed experiments whereby we can manipulate intracellular Ca$^{2+}$ concentration with or without blockade of ATP turnover in rat EDL muscles ex vivo. In short, we demonstrated that we could blunt protein synthesis by ~65% with a sustained raise of intracellular Ca$^{2+}$ and subsequent heightened contraction and energy turnover by treatment of muscles with agents that increase intracellular Ca$^{2+}$. This blunting of protein synthesis was halved when we blocked contraction and ATP turnover, demonstrating that the blunting of protein synthesis is under dual control of Ca$^{2+}$ and energy turnover related signaling (84a).

So, what enzyme(s) could be downstream of Ca$^{2+}$ and energy charge-related signaling leading to altered translation factor activity? Conceivably, the increase in skeletal muscle eEF2 phosphorylation with contractions could result from increased activity of the upstream eEF2 kinase (EF2K) or decreased activity of the upstream eEF2 phosphatase [i.e., protein phosphatase 2A (PP2A)], or a combination of both (13). Earlier work suggested that EF2K was the upstream affector of eEF2 phosphorylation in working skeletal muscle (86) and heart (44), but neither study could rule out the effect of upstream phosphatase activity. However, as also stated above, treatment of isolated rat skeletal muscles with eEF2 kinase inhibitors fully block eEF2 phosphorylation during contractions, and thus it is the activation of eEF2 kinase mediating this effect. Also, many factors which activate eEF2K such as a rise in intracellular Ca$^{2+}$ (57, 68, 88) a fall in pH (27) as well as AMP-activated protein kinase (12, 44, 45, 105) and cAMP-dependent protein kinase (22, 60, 77) activity, are known to be upregulated in contracting skeletal muscle during exercise (Fig. 3; Refs. 29, 61, 96, 106, 107). It may also be that there are decreases in the activity of kinases that phosphorylate eEF2K to decrease its activity, such as mammalian target of rapamycin complex 1 (mTORC1) (106). However, because studies have now shown that in vitro activity of eEF2 kinase is not altered in contracting skeletal muscle (62, 86) it is unlikely that there is a role for altered phosphorylation of eEF2K during contractions. Indeed, the increases in eEF2 phosphorylation and AMPK activity during exercise/contractions have been shown to be dissociated in exercise time-course and intensity experiments, during energy-turnover blockade, and in mice over expressing inactive AMPK (Fig. 3; Refs. 62, 84a, 85, 86). Furthermore, using a phosphospecific antibody (12), our laboratory has been unable to detect eEF2 phosphorylation at the putative AMPK site in skeletal muscle samples (Rose AJ, Proud CG, and Richter EA, unpublished observations) even though skeletal muscle eEF2K has a similar amino acid sequence around this site when compared with eEF2K from tissues that display phosphorylation at this site (62). It is unlikely that lowered pH plays a role because in vitro skeletal muscle eEF2K activity was not different when assayed at pH 6.8 vs. pH 7.2 (84a), and an increase in eEF2 phosphorylation during moderate-intensity exercise occurs within the first minute of exercise (86) at which point muscle pH is unlikely to have decreased appreciably. Moreover, it is questionable whether altered intramuscular pH even affects muscle protein

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\text{Fig. 3. Time effect of exercise/contraction on skeletal muscle eukaryotic}
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\text{elongation factor 2 (eEF2) and 5'-AMP activated protein kinase (AMPK) phosphorylation. Men undertook cycling exercise in the fasted state and muscle samples from the vastus vateralis were collected at rest and at selected time-points during exercise. These samples were processed and analyzed for phosphorylated eEF2 and AMPK using standard Western blotting techniques. AU, arbitrary units; pT, phospho-Thr. Data are reproduced from Rose et al. (86) with permission.}
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Fig. 4. Putative signaling mechanisms in the blunting of skeletal muscle mRNA translation during exercise. The synthesis of proteins involves ribosomal messenger RNA (mRNA) translation. Translation is conventionally divided into three steps: initiation, elongation, and termination, which are catalyzed by eukaryotic initiation (eIF), elongation (eEF), and release factors, respectively. Skeletal muscle eIF4E binding protein 1 (4E-BP1) phosphorylation decreases during exercise, which may act to retard the formation of the eIF4E-eIF4G complex and thus translation initiation. Skeletal muscle eEF2 phosphorylation, which retards eEF2 activity, increases during exercise and may act to retard translation elongation. Signaling upstream of these events is not fully resolved but probably involves decreased mammalian target of rapamycin complex 1 (mTORC1) and increased eEF2 kinase (eEF2K) activity downstream of altered cellular energy charge and intracellular Ca2+ respectively (see text for details). AMPK, 5’AMP-activated protein kinase; CaM, calmodulin. The m7GTP (7-methylguanosine triphosphate) on the mRNA strand represents mature capped mRNA, which is essential for initiation factors to bind.

synthesis during contractions because studies show that protein synthesis is only mildly affected, if at all, by incubation of muscles in media of pH 7.4 vs. pH 7.0 (91). On the other hand, a preliminary report has shown that addition of Ca2+ to skinned skeletal muscle results in inhibition of protein synthesis during contraction (91). This inhibition of protein synthesis is thought to be mediated by an inhibition of eEF2 kinase activity, which is downstream of Ca2+/calmodulin (CaM) blunts protein synthesis in skinned skeletal muscle fibers (47). In addition, because skeletal muscle eEF2 phosphorylation is rapidly increased by exercise/contraction (Fig. 3; Refs. 62, 86) and eEF2K can be selectively activated by Ca2+/CaM in vitro (86, 88), we propose that the inhibition of eEF2 activity by phosphorylation is downstream of Ca2+/CaM-eEF2K signaling cascade (Fig. 4).

Unlike eEF2, the regulation of skeletal muscle 4E-BP1 phosphorylation during exercise is more obscure (Fig. 4). The kinase that phosphorylates 4E-BP1 is mammalian target of rapamycin (mTOR; Ref. 74), and Williamson et al. (106) presented evidence that skeletal muscle mTOR activity decreases in running mice. The mechanism behind reduced mTOR activity was not entirely clear, but because increases in AMP-activated protein kinase (AMPK) activity seem to correlate, albeit rather weakly, with decreases in 4E-BP1 phosphorylation in skeletal muscle during exercise (54, 85, 106), this could be a potential mechanism. Indeed, several studies have now shown that treatment of muscles with a chemical activator of AMPK can decrease 4E-BP1 phosphorylation and that AMPK is indeed the kinase responsible for the effect (8, 21, 95, 105). However, we and others (28) have observed dissociations between changes in AMPK activity and 4E-BP1 phosphorylation. In particular, our laboratory (84a) has shown that the dephosphorylation of skeletal muscle 4E-BP1 was normal during contractions in mice overexpressing an inactive form of AMPKα2 in muscles. However, given that there are many lines of evidence indicating that energy charge can regulate cellular protein synthesis rate (14, 44, 57) it may be that AMPK or a similar signaling event could negatively regulate the mRNA translation machinery and further studies are warranted to investigate this.

Another downstream target of mTORC1 is p70s6 kinase 1 (S6K1; Ref. 74). S6K1 phosphorylates ribosomal S6 proteins and perhaps other substrates, the functional consequence of which is not fully understood (74). Nonetheless, because increases in S6K1 activity correlate well with increases in skeletal muscle protein anabolism (4, 94), it may be that decreases in S6K1 activity in working muscle may be part of the mechanism of blunted muscle protein synthesis during exercise. However, despite some studies showing that there can be potent increases in phosphorylation of human skeletal muscle S6K1 at Thr421/424 during endurance (59) and resistance (20, 54) exercise, other studies report no change in the in vitro activity of S6K1 (92) or phosphorylation of S6 (18, 54, 62, 104) during contractile activity. This is probably because phosphorylation of S6K1 at this Thr421/424 site alone is important in terms of intracellular kinase activity (74). On the other hand, S6K1 phosphorylation at Thr389, a potent regulatory site for intracellular kinase activity (74), is unaltered or perhaps increased (104) during exercise/contractile activity. In any case, S6K1 activity is not lower and thus would not contribute to the suppression of muscle protein synthesis during exercise.

ARE THERE DIVERGENT RESPONSES WITH DIFFERENT EXERCISE MODES?

As it stands now, there are no clear-cut data showing that resistance-type and endurance-type exercise affect skeletal muscle protein synthesis rates differently, when examined during exercise. Results of separate studies suggest that both resistance and endurance exercise both result in reduced muscle protein synthesis (14, 16, 25, 28, 62, 70) during the exercise bout. However, it was recently shown that there are divergent
effects of resistance- and endurance-type stimulation on skeletal muscle translation factor phosphorylation ex vivo (3), but unfortunately, protein synthesis was not selectively measured during the stimulation period. However, a more recent study of humans (104) showed that, in untrained individuals, there was no striking divergence between skeletal muscle signaling proteins immediately after resistance- and endurance-type exercise. Similar to carbohydrate and fat metabolism (40, 83, 99), it could be that the intensity of exercise/contraction could be an important determinant of the magnitude of the depression of protein synthesis during exercise. In vivo, the extra force generated when moving from low-intensity exercise to high-intensity exercise is a combination of greater recruitment of type II muscle fibers and a greater stimulation rate of already recruited fibers (90). On this, Bylund-Fellenius et al. (14) showed that fast- but not slow-twitch muscle exhibited decreases in protein synthesis rate when undergoing contraction. Our laboratory also has unpublished data showing that the magnitude of depression of protein synthesis is greater in fast-twitch EDL vs. slow-twitch soleus rodent muscles when stimulated to contract using identical protocols ex vivo (84a). Furthermore, we have shown that the magnitude of suppression of protein synthesis is greater when fast-twitch muscles were stimulated to contract ex vivo with a high-intensity vs. low-intensity protocol (84a). In humans, there were similar increases in eEF2 but higher 4EBP1 dephosphorylation in skeletal muscle during high- vs. low-intensity cycling exercise (85). Altogether, these results suggest that there may be a role for exercise intensity and perhaps also duration (11) on the magnitude of suppression of protein synthesis rate during exercise, perhaps explained by different responses or signaling in different fiber types; however, this remains to be formally tested in vivo and in humans. Lastly, because in situ lengthening contractions actually increase skeletal muscle 4E-BP1 and S6K1 Thr\(^{389}\) phosphorylation (31, 66, 95) then there may be differences in muscle protein synthesis when undergoing lengthening vs. shortening contractile activity.

**REGULATION OF SKELETAL MUSCLE PROTEIN BREAKDOWN DURING EXERCISE**

Whether there are changes in protein breakdown in working skeletal muscle is controversial. Earlier studies using 3-methylhistidine release as a marker for myofibrillar breakdown showed that myofibrillar protein breakdown may be lower than basal conditions in rats subjected to running (26) or to contractions in situ (14). On the other hand, there was no change in total protein breakdown during mild or intense muscle contractions, but myofibrillar protein breakdown was higher during intense contractions of rat skeletal muscle ex vivo (69). However, it should be noted that the use of 3-methylhistidine release as a marker of myofibrillar protein breakdown may be invalid (78). In humans, studies examining the balance of noncatabolized amino acids across working limbs have shown that there is a net release of the amino acids during exercise (36, 37, 97, 98), which appears to be most evident when exercise intensity is high (7, 98). Indeed, total and nonmetabolizable amino acid release from working skeletal muscle is high in individuals with McArdle’s disease (102). These individuals cannot use muscle glycogen as a substrate for ATP turnover during exercise and consequently exhibit a much greater metabolic stress during exercise than normal controls (102). Thus the general consensus is that amino acid release occurs during exercise in working skeletal muscle but only when exercise intensity is quite heavy (81, 101). Some authors have attributed this release of amino acids to be indicative of enhanced protein breakdown (101), but as surmised by Kumar et al. (56) in this review series, this amino acid release may simply reflect a greater magnitude of suppression of protein synthesis relative to a suppression of protein breakdown. It is our opinion that, perhaps due to limitations of methods, there is no study that has accurately assessed whether there are changes in skeletal muscle protein breakdown during exercise and that further studies, perhaps using pulse-chase tracer methods, are clearly required to clarify this issue. For further discussion of this topic see review by Rennie and coworkers (56) in this review series.

**REGULATION OF PROTEOLYSIS**

Skeletal muscle protein breakdown occurs by a variety of mechanisms including lysosomal, ATP-dependent ubiquitin system, ATP-independent ubiquitin system, and calcium-dependent proteases (52). Very few studies have examined possible changes to these systems in skeletal muscle during exercise. Despite some studies reporting higher lysosomal activity in skeletal muscle after exercise (26), no study has investigated this during exercise. As reviewed by Reid (82), chronic physical activity has a clear effect on the skeletal muscle ubiquitin-proteasome activity, and that myogenic regulatory factor myoD (myoD) degradation and higher nuclear factor-κB (NF-κB) activity can be used as surrogate markers of the activity of this pathway. Although no study has examined skeletal muscle myoD expression in relation to exercise, higher NF-κB activity has been reported in rodents (43, 50) and lower NF-κB activity has been reported in humans (30) in skeletal muscle after exercise. Thus, at this stage, we believe that there is no clear consensus on the activation of the ubiquitin-proteasome pathway in skeletal muscle during exercise, and further work is required to determine this. Lastly, studies show that raising intracellular Ca\(^{2+}\) in skeletal muscles ex vivo can stimulate protein breakdown (5, 34, 51, 109), which probably involves nonlysosomal proteolysis (109) involving thiol-proteases (5), and selective breakdown of nonmyofibrillar proteins (34). Thus an obvious candidate for the action of Ca\(^{2+}\) to stimulate protein breakdown would be the Ca\(^{2+}\)-activated proteases. However, recent work has shown that calpains are not activated in skeletal muscle of humans during exercise (64), probably because the Ca\(^{2+}\) concentration during normal excitation is not sufficient (19, 65). Lastly, others (14) have hypothesized that lower myofibrillar protein degradation in skeletal muscle during contractile activity may be mediated by energy depletion and metabolite accumulation.

**WHAT IS THE FUNCTIONAL SIGNIFICANCE OF ALTERED PROTEIN TURNOVER DURING EXERCISE?**

So what could be the functional significance, if at all, of blunted skeletal muscle protein synthesis during exercise? Some authors (13) have suggested that because protein synthesis requires a lot of energy that the switching off of this process during cellular stress could conserve energy for the other, more pivotal processes. Although this may hold true for other tissues, it is unlikely to be so for skeletal muscle, because...
protein turnover is relatively slow in this tissue and the energy required for this process is relatively minor compared with the massive ATP turnover consumed by the activated ATPases (i.e., myosin-ATPase, Ca\(^{2+}\)-ATPase, Na\(^-\)K\(^{+}\)-ATPase) in contracting skeletal muscle. Nonetheless, the reduction in this relatively minor ATP turnover due to protein synthesis, and perhaps other anabolic pathways, may allow muscles to contract slightly more efficiently, although this remains to be formally tested. On the other hand, others (23) have hypothesized that the blunting of protein synthesis leaves the free amino acid pool available for catabolic processes. In addition, it has been suggested that activation of proteases during strenuous activity may contribute to long-lasting fatigue (71), although this remains to be proven (2). Lastly, it was originally hypothesized by Pain and Manchester (70) that “It seems unlikely therefore that protein synthesis will be enhanced during exercise in vivo, but rather the reverse, and the increase in protein formation after exercise is more probably a compensatory response to an initial decline than a result of exercise per se.” Later, Bylund-Fellenius et al. (14) speculated that “...the mechanism for net protein synthesis postexercise is triggered by these acute changes” during exercise. Because it is likely that the higher skeletal muscle protein synthesis after exercise is due to enhanced mRNA translation (75, 78) probably involving the mTOR pathway (9, 75), this hypothetical “mechanism” should involve this pathway. Recently, it was shown that mTORC1 activity and protein synthesis are actually enhanced following a few hours of chemical inhibition of mRNA translation elongation in cells and that this was due to a selective reduction in the expression of the mTORC1 inhibitor protein, regulated in development and DNA damage responses [regulated in development and DNA damage responses (REDD)] 1 (48). Thus, because REDD isoforms are expressed in skeletal muscle (103), we hypothesize that a putative decrease in skeletal muscle REDD by the blunting of mRNA translation during exercise is part of the molecular mechanism by which prior exercise enhances muscle mTORC1 activity and protein synthesis.

SUMMARY AND CONCLUSIONS

In summary, during exercise, catabolism of amino acids contributes very little to ATP turnover in working muscle. With regard to protein turnover, there are now consistent data from tracer studies in rodents and humans showing that global protein synthesis is blunted in working skeletal muscle. Whether there is altered skeletal muscle protein breakdown during exercise remains unclear. The blunting of protein synthesis is believed to be mediated by suppressed mRNA translation initiation and elongation steps involving, but not limited to, changes in 4E-BP1 and eEF2, respectively. Upstream signaling to translation factors is mediated by changes in signaling downstream of intracellular Ca\(^{2+}\) and altered energy turnover, and a signaling cascade involving Ca\(^{2+}\)/CaM-eEF2K-eEF2 is likely to be important. However, the roles of other signaling mechanisms, such as mechanotransduction, oxidative stress, altered redox state, and perhaps autocrine/paracrine factors, have not been studied and may be involved. Recent studies examining protein turnover have been able to tease out different responses of different protein pools (104) and even specific proteins (46), and it is envisaged that these, and perhaps other methods (67) as well as studies using genetic manipulation of cells and animals, can aid in the understanding of skeletal muscle protein turnover during exercise. The knowledge of such is not just important from a physiological perspective but also may lead to development of new treatments for individuals with muscle dysfunction.

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PROTEIN METABOLISM DURING EXERCISE


