Availability of eIF4E regulates skeletal muscle protein synthesis during recovery from exercise

Gautsch, T. A., J. C. Anthony, S. R. Kimball, G. L. Paul, D. K. Layman, and L. S. Jefferson. Availability of eIF4E regulates skeletal muscle protein synthesis during recovery from exercise. Am. J. Physiol. 274 (Cell Physiol. 43): C406–C414, 1998.—We examined the association of the mRNA cap binding protein eIF4E with the translational inhibitor 4E-BP1 in the acute modulation of skeletal muscle protein synthesis during recovery from exercise. Fasting male rats were run on a treadmill for 2 h at 6 m/min and were reallimented immediately after exercise with either saline, a carbohydrate-only meal, or a nutritionally complete meal (54.5% carbohydrate, 14% protein, and 31.5% fat). Exercised animals and nonexercised controls were studied 1 h postexercise. Muscle protein synthesis decreased 26% after exercise and was associated with a fourfold increase in the amount of eIF4E present in the inactive eIF4E·4E-BP1 complex and a concomitant 71% decrease in the association of eIF4E with eIF4G. Refeeding the complete meal, but not the carbohydrate meal, increased muscle protein synthesis equal to controls, despite similar plasma concentrations of insulin. Additionally, eIF4E·4E-BP1 association was inversely related and eIF4E·eIF4G association was positively correlated to muscle protein synthesis. This study demonstrates that recovery of muscle protein synthesis after exercise is related to the availability of eIF4E for 48S ribosomal complex formation, and postexercise meal composition influences recovery via modulation of translation initiation.

eukaryotic initiation factor 4E; rats; isoleucine; translation initiation; 4E-BP1

A DEPRESSION IN THE fractional rate of skeletal muscle protein synthesis occurs with prolonged exercise (7, 8). Changes in protein turnover after exercise suggest that recovery from exercise occurs through stimulation of protein synthesis (29); however, the mechanisms for recovery of skeletal muscle protein synthesis after exercise are currently unknown. It is generally accepted that short-term changes in protein synthesis are achieved at the translation level, and, in many situations, it is thought that the rate of initiation limits overall translation (24). Hence, it has been suggested that recovery of muscle protein synthesis after exercise occurs at the level of translation initiation (25), although there are no reports to confirm this hypothesis.

The initiation phase of protein synthesis is an important site of translational control, for it allows the cell to alter protein synthesis rates rapidly and to reverse any alteration equally as rapidly (15). Recent evidence from several investigators has indicated that binding of the mRNA to the 43S preinitiation complex is a rate-controlling step in translation initiation (19, 32). This step requires the group four eukaryotic initiation factors (eIFs), eIF4B and eIF4F. eIF4F is a three subunit complex consisting of 1) eIF4A, a RNA helicase that functions in conjunction with eIF4B to unwind secondary structure in the 5' untranslated region (UTR) of the mRNA; 2) eIF4E, a protein that binds the mRNA cap structure and is important for selection and stability of the mRNA to be translated; and 3) eIF4G, a 220-kDa polypeptide that is suggested to function as a scaffold for eIF4E, eIF4A, the mRNA, and the ribosome (via association with eIF3) (32). The eIF4F complex collectively serves to recognize, unfold, and guide the mRNA to the 43S preinitiation complex.

Alternations in either the phosphorylation state or in the availability of eIF4E for binding eIF4G appear to modulate the function of the eIF4F complex. Phosphorylation of eIF4E has been suggested to increase translation rates via increased association with eIF4G and eIF4A (22) and/or increased mRNA cap-binding affinity (21). Conversely, the availability of eIF4E for eIF4F complex formation appears to be modulated by the translational inhibitory phosphoprotein 4E-BP1 (27). 4E-BP1 competes with eIF4G for binding eIF4E and has the ability to physically sequester eIF4E into an inactive complex. Changes in the relative affinity of eIF4E for eIF4G vs. 4E-BP1 due to phosphorylation of one or more of these proteins is postulated to regulate rates of translation.

Few studies have examined the effect of food intake on recovery of muscle protein synthesis. Recent data demonstrate that consumption of a complete meal after acute food deprivation stimulates muscle protein synthesis via increases in initiation activity (36), whereas other findings suggest that a combination of insulin plus amino acids is required for stimulation of muscle protein synthesis (28). However, to our knowledge, no data exist examining the influence of postexercise meal composition on recovery of skeletal muscle protein synthesis.

The objectives of the present study were twofold: 1) to examine the influence of postexercise feeding and meal composition on the recovery rate of muscle protein synthesis, and 2) to investigate if changes in muscle protein synthesis with postexercise feeding and/or meal composition can be attributed to changes in translation initiation via alterations in eIF4E phosphorylation or availability. This study demonstrates that inhibition of muscle protein synthesis after prolonged exercise is attributed to reduced availability of eIF4E. Feeding a nutritionally complete meal, but not a carbohydrate meal, resulted in a complete recovery of muscle protein...
present study indicated that this animal population con-
sumed 293 ± 9 kJ/body/day (n = 20); therefore, the experimen-
tal meals supplied ~15% of daily energy intake for this age and
carbohydrate alone or a complete macronutrient mixture to stimu-
late protein synthesis during recovery. The study had five treat-
ment groups (n = 4 per group): CF, nonexercised controls
exercised and fed a nutritionally complete meal after the
experimental run; EEm, exercised and fed a 100%
carbohydrate meal after the experimental run; and EEm,
exercised and fed a nutritionally complete meal after the
experimental run. All animals were allowed free access to water after
the treadmill bout, but no food was available after exercise
beyond the defined postexercise meals. Fed controls (CM
group) intubated with the nutritionally complete meal were
included in the study design to determine any parallel effects of the food deprivation on muscle protein synthesis.

The experimental run was performed on the ninth day and
consisted of 2 h of treadmill running at 26 m/min (1.5% grade).
Previous reports on energy expenditure in this strain of rat
indicated that this exercise intensity corresponds to ~75% max-
imum oxygen consumption (31). Exactly 1 h after the termina-
tion of the experimental run, animals were anesthe-
tized by CO2 and killed by decapitation. The decision to
investigate muscle recovery at 1 h after exercise was based on
previous work in rodents which showed realimentation after
food deprivation to increase muscle protein synthesis within
1 h of refeeding (10, 20, 37). Trunk blood was collected and
centrifuged at 1,800 g for 10 min to obtain plasma.

Plasma measurements. Plasma glucose was analyzed by a
glucose oxidase-peroxidase automated method (YSI model
2300 analyzer, Yellow Springs Instruments, Yellow Springs,
OH). Plasma corticosterone and plasma insulin were ana-
yzed using commercial RIA kits.

Administration of metabolic tracer. A bolus dose (0.7 ml/
100 g body wt) of L-[4,5-3H]isoleucine (200 mM containing 130
µCi/ml) was injected via the tail vein 50 min after the end of
exercise for the in vivo measurement of skeletal muscle
protein synthesis (11). The right gastrocnemius and plantaris
were excised as a unit 10 min after injection and quickly
frozen in liquid nitrogen for storage in a −80°C freezer.
The elapsed time from injection until freezing of muscle in
liquid nitrogen was recorded as the actual infusion time.

Muscle isoleucine specific radioactivity. Frozen muscle was
powdered under liquid nitrogen with a mortar and pestle. A
100-µl aliquot was combined with 1.25 ml extraction buffer
(40 mM sodium pyrophosphate, 20 mM sodium phosphate,
and 0.5 mM EDTA), then homogenized using a Polytron
(Kinematica, Krien-Luzern, Switzerland). After homogeniza-
tion, 150 µl of 100 g/l sodium dodecyl sulfate were added, and
the sample was boiled for 3 min and then centrifuged 10 min
at 1,000 g. Soluble protein was precipitated from a 100-µl
aliquot of the supernatant by adding 1.25 ml of cold 100 g/l
trichloroacetic acid and refrigerating overnight. The sample
was centrifuged for 3 min, and the resulting pellet was
washed three times with 1 ml of 50 g/l trichloroacetic acid
and once with 0.5 ml of 100% ethanol. The pellet was rinsed
with 50 µl acetone and air dried. The dry pellet was transferred to
a hydrolyzing ampule combined with 1 ml of 6 M HCl, flame
sealed, and placed in a 110°C oven for 24 h. The solution was
transferred into 12 × 75-mm glass tubes and evaporated in a
speed vacuum (Savant Instruments, Farmingdale, NY). The
pellet was rinsed with 1 ml of distilled water and rehydrated.
The resulting pellet was reconstituted in 750 µl of 1.5 mM
norleucine in distilled water. Ten milliliters of scintillation
cocktail were added to a 350-µl aliquot, and disintegrations
per minute were calculated from quenched standards (model
LS9000 Liquid Scintillation Counter, Beckman Instruments,
PaloAlto, CA). Sample isoleucine concentration (pmol/µl) was
determined by high-performance liquid chromatography
(HPLC) by derivatizing a 10-µl aliquot with phenylisothio-
cyanine (26).

Intracellular and plasma free isoleucine specific radioactivity.
One milliliter of low-salt buffer (40 mM NaCl, 5 mM
NaPO4, at a pH of 7 by mixing di- and monobasic forms, 1 mM
MgCl2, 0.1 mM dithiothreitol, and 0.1 mM EDTA) was added
to 100 mg powdered muscle. The sample was mixed vigor-
ously on a vortex for 45 s and then centrifuged for 3 min at
4°C. Amino acids were separated from proteins by filtering
400 µl of supernatant or 400 µl plasma through a 10,000
nominal molecular weight limit filter (Millipore, Milford, MA)
at 4°C. A 50-µl aliquot of the muscle-derived filtrate or a 20-µl aliquot of the plasma-derived filtrate was filtered over a neutralized cation exchange column (50 WX4-400, Sigma Chemical, St. Louis, MO), rinsed three times with 1 ml distilled water, then eluted with 2 ml of 25% (vol/vol) NaOH. The solutions were evaporated in a speed vacuum as before. After solutions were reconstituted in 110 µl distilled water, a 50-µl aliquot of each was derivatized with phenylisothiocyanate and reconstituted in 100 µl of diluent (Pico-Tag sample diluent for free amino acid analysis, Waters Chromatography, Milford, MA). A 30-µl aliquot of each sample was combined with 10 ml scintillation cocktail for the determination of radioactivity. The isoleucine concentration in 30-µl aliquots of remaining diluent containing muscle free amino acids and 10-µl aliquots of remaining diluent containing plasma free amino acids were measured by HPLC. Specific activity was calculated from the radioactivity divided by the amount of isoleucine. All specific activity measurements were performed in duplicate.

Calculation of the fractional rate of protein synthesis. Protein synthesis was calculated as a fractional rate of synthesis (K2, %/day) according to McNurlan et al. (18): K2 = (SI × 100)/(SF × t), where SI represents isoleucine specific activity incorporated into muscle protein, SF represents the intracellular free pool isoleucine specific activity, and t represents the infusion time in days.

Quantitation of 4E-BP1·eIF4E and eIF4G·eIF4E complexes. Phosphorylation of p70S6K. Phosphorylation of p70S6K was determined in 10,000-g supernatants on a slab gel by 10.2 ± 0.33.5 on June 22, 2017 http://ajpcell.physiology.org/ Downloaded from

RESULTS

Preliminary experiments were performed with food-deprived animals to validate use of isoleucine for the flooding dose technique (11). Underlying assumptions for an amino acid tracer are as follows: 1) the specific activity of free isoleucine in plasma and muscle is constant and equivalent to the infused dose during the experimental period, and 2) the rate of incorporation of labeled isoleucine into muscle protein increases over time. Figure 1A demonstrates that the specific activity of free isoleucine was constant over 15 min and accounted for >85% of the total infused specific activity. A linear increase of labeled isoleucine (r2 > 0.9) incorporated into skeletal muscle protein is shown in Fig. 1B.

Fig. 1. Time course of specific radioactivities of free (A) and protein-bound (B) isoleucine in plasma and skeletal muscle (gastrocnemius and plantaris) in rats fasted for 10 h. A flooding dose of isoleucine (200 µmol · kg−1 · isoleucine with 4.77 MBq · [4,5,5-3H]isoleucine) was administered via tail vein. Values are means ± SE; n = 3 animals per point. Infused dose is equal to 100% specific radioactivity.
Prolonged exercise did not depress plasma glucose (Fig. 2) or insulin (Fig. 3) concentrations below the nonfed controls. Feeding the carbohydrate meal immediately after exercise resulted in a significant increase in plasma glucose at 1 h postexercise that was not seen with feeding the complete meal. Conversely, insulin concentrations increased similarly in response to feeding either the carbohydrate or the complete meal (P < 0.06 for both EC vs. EF and EM vs. EF). Plasma corticosterone was increased in the EF and EC groups above meal-fed controls (Fig. 4) and tended to be increased above food-deprived controls (P < 0.07). Surprisingly, animals fed the complete meal after exercise had plasma corticosterone levels 54% below the other exercise groups and were not different from controls.

Skeletal muscle protein synthesis was depressed 26% in animals not fed after exercise compared with baseline (CF group) (Fig. 5). This depression cannot be attributed to the exercise protocol and not solely lack of food, for muscle protein synthesis in the EF group was not different from the nonexercised fed condition (CM group). One hour after exercise, muscle protein synthesis in the carbohydrate-fed group was not significantly stimulated above animals not fed after exercise. In contrast, muscle protein synthesis rates in exercised animals fed the complete meal were increased compared with the nonfed and carbohydrate-fed animals and were recovered to rates that were not different from baseline. The enhanced rate of recovery cannot be attributed to a differential insulin response, for insulin concentrations in the carbohydrate and complete meal groups were similar. Interestingly, plasma cortico-

**Fig. 2.** Plasma glucose in male rats 1 h after 2-h experimental run. Values are means ± SE; n = 4 animals. CF, food-deprived controls; CM, meal-fed controls; EF, exercised and food-deprived postexercise; EC, exercised and fed a 100% carbohydrate meal postexercise; EM, exercised and fed a complete meal postexercise. Means not sharing same superscript(s) are different (P < 0.05).

**Fig. 3.** Plasma insulin in male rats 1 h after 2-h experimental run. Values are means ± SE; n = 4 animals. Definitions are as in Fig. 2. Means not sharing same superscript(s) are different (P < 0.05).

**Fig. 4.** Plasma corticosterone in male rats 1 h after 2-h experimental run. Values are means ± SE; n = 4 animals. Definitions are as in Fig. 2. Means not sharing same superscript(s) are different (P < 0.05).

**Fig. 5.** Fractional rate of skeletal muscle protein synthesis (K_s, %/day) in male rats 1 h after 2-h experimental run. Values are means ± SE; n = 4 animals. Definitions are as in Fig. 2. Means not sharing same superscript(s) are different (P < 0.05).
rone concentrations were inversely related to muscle protein synthesis ($r = -0.77, P < 0.01$).

One mechanism through which muscle protein synthesis is depressed is via diminished availability of eIF4E for eIF4F active complex formation, inhibiting translation initiation. Decreased eIF4E availability occurs via 4E-BP1 sequestering eIF4E into an inactive complex, preventing eIF4G binding eIF4E (13). On an immunoblot, this is visualized as an increase in the amount of 4E-BP1 present in the eIF4E immunoprecipitate. Accompanying the depression in muscle protein synthesis, 4E-BP1·eIF4E complex formation was increased fourfold after prolonged exercise (Fig. 6). An increase in eIF4E binding to 4E-BP1 implies a reduced availability of eIF4E to associate with eIF4G, based on in vivo data which suggest that eIF4E cannot bind both eIF4G and 4E-BP1 concurrently (17). In accordance with this report, the amount of eIF4G present in the eIF4E immunoprecipitate was decreased 71% (Fig. 7). These data suggest that after prolonged exercise, eIF4F function was abated by a decrease in eIF4E availability, which led to an inhibition of muscle protein synthesis.

In accordance with this mechanism, recovery of muscle protein synthesis at the level of translation initiation level would therefore occur via increased availability of eIF4E for eIF4F active complex formation, stimulating translation initiation. Increased eIF4E availability occurs via the dissociation of the eIF4E·4E-BP1 inactive complex, allowing the released eIF4E to interact with eIF4G (13). Consistent with the fractional synthesis rate, consumption of the carbohydrate meal did not significantly alter the association between 4E-BP1 and eIF4E (Fig. 6) or the amount of eIF4G present in the eIF4E immunoprecipitate (Fig. 7). In contrast, the exercise-induced increase in 4E-BP1·eIF4E association was completely abated in exercised animals fed the complete meal, and eIF4G·eIF4E association was increased fivefold above the nonfed exercised animals. The pattern of 4E-BP1·eIF4E complex formation during recovery was inversely related to the fractional rate of skeletal muscle protein synthesis ($r = -0.65, P < 0.01$). Additionally, the pattern of eIF4G·eIF4E complex formation was positively related ($r = 0.71, P < 0.01$) to the fractional rate of skeletal muscle protein synthesis. These results support a role for eIF4E availability in the regulation of muscle protein synthesis during exercise recovery.

To further investigate the mechanism through which eIF4F function is altered, changes in the phosphorylation state of eIF4E were examined. The phosphorylation of eIF4E in vitro has been shown to be increased under a variety of conditions where translation rates are enhanced (32). Almost all of the eIF4E present in muscle of exercised rats was in the phosphorylated state (>90%), with the proportion not differing among exercise groups (Fig. 8). In contrast, the phosphorylation of eIF4E in the meal-fed controls was 60% less than nonfed controls ($P < 0.018$).
It has been suggested that regulation of translation initiation might occur via the p70S6K signaling pathway. The p70S6K signaling pathway is involved in the phosphorylation of the ribosomal protein S6 and has been implicated in stimulating protein synthesis and increasing 4E-BP1 phosphorylation (32). The phosphorylation of the p70S6K is associated with its activation. On a polyacrylamide gel, this results in phosphoforms that exhibit decreased electrophoretic mobility when subjected to electrophoresis. There were no differences in the phosphorylation of p70S6K between the exercised animals and the nonfed controls (Fig. 9). However, meal-fed controls showed an increase in the phosphorylation of the kinase above the nonfed controls.

**DISCUSSION**

It has been recognized for over a decade that the primary change in muscle protein turnover during and immediately after endurance exercise is a depression in muscle protein synthesis (7), with the magnitude of depression dependent on the intensity and duration of exercise (2, 8). Additionally, prior exercise training has no effect on increasing protein synthesis or reducing degradation after prolonged exercise (7). Although the acute effect of prolonged exercise on protein turnover is catabolic, routine exercise programs do not lead to muscle atrophy. Physical activity and exercise training are associated with maintenance or enlargement of skeletal muscles; therefore, at some point during recovery from endurance exercise, increases in protein synthesis and/or decreases in protein degradation must occur. Carraro et al. (6) showed that protein synthesis values in humans were elevated 22% 4 h after exercise as compared with nonexercise conditions. Similarly, Rennie et al. (29) demonstrated synthesis rates in human subjects to increase 22% above the rested state 4.5 h after 3.75 h of treadmill exercise. Taken together, protein turnover changes after exercise suggest that the protein catabolic state induced by exercise continues beyond the end of exercise and that recovery from endurance exercise is ultimately driven by increases in protein synthesis.

Protein synthesis rates are mediated by extracellular signals such as nutrients and hormones. One of the signals that may mediate the depression in muscle protein synthesis during and after endurance exercise is insulin. Studies using perfused hindlimb preparations and incubated muscles have established an essential role for insulin in the maintenance of muscle protein synthesis (15), while the absence of insulin in the form of streptozotocin-induced diabetes causes extreme muscle catabolism in rats due to decreases in protein synthesis (9). The ability of insulin to stimulate glucose and amino acid uptake is enhanced after exercise (37); however, the role of insulin in the regulation of muscle protein synthesis after exercise is unclear. Balon et al. (2) found the ability of 200 µU/ml insulin to stimulate protein synthesis in perfused rat muscles was not enhanced after high- or moderate-intensity exercise. Additionally, the results of the current study suggest that low insulin concentrations cannot be solely responsible for the depression in protein synthesis after exercise, for insulin concentrations between the sedentary, food-deprived animals and the exercised, food-deprived animals were similar.

Studies investigating the refeeding period after short-term fasting have also identified insulin as an extracellular signal that mediates increases in muscle protein synthesis. Injection of anti-insulin serum before food intake suppresses the increase in muscle protein synthesis observed with refeeding (28). This finding is in accordance with Yoshizawa et al. (36) and Svanberg et al. (34), who found that administration of insulin...
antibodies to fasting mice prevents the stimulatory effect of refeeding a compete meal on muscle protein synthesis. These studies establish the importance of insulin in mediating the acute stimulation of muscle protein synthesis during refeeding. However, Garlick et al. (10) demonstrated that only supraphysiological concentrations of insulin could mimic the response in muscle protein synthesis seen with refeeding when infused in postabsorptive rats. Additionally, exogenous insulin given to freely fed mice does not further enhance muscle protein synthesis (34). These findings suggest that insulin is not solely responsible for the rise in muscle protein synthesis by food intake. Thus, instead of directly modulating the biosynthetic process after exercise, insulin might play a permissive role in the regulation of muscle protein synthesis.

Recent work in mice shows that refeeding a nutritionally complete meal after an overnight fast stimulates muscle protein synthesis, but not when mice are refed a protein-free meal (36). This effect is seen despite insulin concentrations not differing between meal conditions and plasma glucose being higher in animals refed the protein-free meal. The authors conclude that amino acids in addition to insulin are required for recovery of muscle protein synthesis with refeeding. The results of the current study concur with this proposal and support the hypothesis of Preedy and Garlick (28) that an increase in muscle protein synthesis by food intake is mediated by insulin acting in cooperation with amino acids. These studies therefore suggest a functional role for amino acids in enhancing the recovery of skeletal muscle protein synthesis by feeding postexercise.

Another possible extracellular mediator in the acute modulation of muscle protein synthesis during recovery from exercise is corticosterone. Corticosterone is the major glucocorticoid produced in the rat and is secreted under conditions of low blood glucose and/or high stress (4). Plasma levels of corticosterone are reported to be elevated after acute exercise (35); however, there are no reports describing the effects of corticosterone on protein synthesis during or after exercise. Administration of corticosterone to streptozotocin-induced diabetic rats for 5 days depressed muscle protein synthesis and RNA activity (the rate of protein synthesis per unit of RNA), suggesting impaired translation initiation (23). The mechanism for the inhibitory effects of corticosterone is unclear but appears to be dependent on low concentrations of insulin. The current data suggest that, under the condition of low insulin, elevated corticosterone levels may further contribute to the inhibition of protein synthesis seen after prolonged exercise.

Corticosterone concentrations are recognized to decline in food-deprived animals upon refeeding, decreasing significantly within an hour of initial food intake (20, 28). Millward et al. (20) refeed food-deprived rats pretreated with anti-insulin serum or corticosterone and determined that the acute increase in muscle protein synthesis by refeeding requires insulin, a fall in corticosterone, and at least one other independent factor. In the current study, animals fed the complete meal after exercise had increased plasma concentrations of insulin and reduced plasma concentrations of corticosterone. In light of the present findings and the available refeeding reports, it is attractive to suggest a role for diminished corticosterone levels acting in concert with elevated insulin and amino acids in mediating recovery of muscle protein synthesis.

Molecular mechanisms for the control of muscle protein synthesis during and after exercise are characterized. mRNA concentrations are unchanged during the relatively brief period of the exercise bout (25), implying regulation occurring at the translation level. The rate of initiation is thought to limit overall translation under most physiological conditions (24), and studies investigating recovery of protein synthesis after acute food deprivation demonstrate increases in polysome size and initiation activity (36). However, to date, there are no studies examining translation initiation during recovery from endurance exercise.

One of the major sites of regulation in the initiation process involves the recognition and unwinding of the mRNA to allow binding to the 40S ribosome. This tightly regulated step requires the participation of eIF4B and eIF4F, a three-subunit complex consisting of eIF4A, eIF4E, and eIF4G (19). One of the most studied factors in this regard is eIF4E, a 24-kDa subunit of the eIF4F complex which serves to select for the mRNA to be translated by binding the m7GTP cap at the 5' end of the mRNA. Binding of eIF4E to the mRNA cap structure is succeeded by its associating with eIF4G, a protein that facilitates the formation of the eIF4F complex and the association of the mRNA with the 40S ribosome (32). The association between eIF4E and eIF4G is important for stabilizing the interaction between the mRNA and the 40S ribosome and is therefore essential for successful 48S preinitiation ribosomal complex formation.

There are currently two characterized mechanisms in which changes in translation initiation occur as a result of altered eIF4F function. The first mechanism involves modulation of eIF4E availability via the binding of eIF4E to the translational repressor, 4E-BP1. Studies in vivo show 4E-BP1·eIF4E association to increase in skeletal muscle of diabetic rats (16) and overnight-fasted mice (33). Both these conditions demonstrate decreases in the phosphorylation state of 4E-BP1 and in eIF4G·eIF4E complex formation, supporting the suggestion that the binding of 4E-BP1 and eIF4G to eIF4E is mutually exclusive and regulated by 4E-BP1 phosphorylation. In the current study, 4E-BP1·eIF4E complex formation was increased, and the amount of eIF4G present in the eIF4E immunoprecipitate was decreased after prolonged exercise, similar to the regulations seen during diabetes and starvation. On the basis of these studies, the effect of exercise on inhibiting translation initiation is most likely due to a decrease in the phosphorylation of 4E-BP1, although alternative explanations for the observed changes, such as modulation of the activity of eIF2B, cannot be ruled out at this time.

The extracellular signal(s) mediating the differential effect of eIF4E availability by refeeding during recovery...
That purified p70S6K does not directly phosphorylate eIF4E, most likely not the kinase that is directly responsible for stimulating muscle protein synthesis in postabsorptive rats, suggesting that changes in muscle protein synthesis with feeding involve more than just insulin. In addition, refeeding both normal and diabetic mice after 18 h of food deprivation induces similar changes in 4E-BP1 phosphorylation and in the association of 4E-BP1 and eIF4G with eIF4E (33), again suggesting that insulin is not required for stimulating translation initiation by refeeding. The current study supports these findings and further suggests that insulin must act in concert with other exogenous signals (such as dietary amino acids or low corticosterone concentrations) to enhance recovery of skeletal muscle protein synthesis after exercise.

A second mechanism of altered eIF4E function involves modulation of the phosphorylation of eIF4E. Studies in culture demonstrate that increases in the phosphorylation of eIF4E by addition of hormones and growth factors lead to increased mRNA cap binding affinity and/or increased association with eIF4G (5, 21), leading to increases in protein synthesis and cell growth. However, in vivo reports do not support results in cell culture, because insulin administration is reportedly associated with a decrease of phosphorylated eIF4E in perfused skeletal muscle (17). Additionally, the amount of eIF4E present in the phosphorylated form does not change during overnight fasting or refeeding (33). In the present study, neither prolonged exercise nor recovery from exercise altered the phosphorylation state of eIF4E in muscle. Furthermore, eIF4E phosphorylation was diminished in the meal-fed rats. This result may be because of increased plasma insulin concentrations alone does not relieve inhibition of skeletal muscle translation initiation after exercise. We suggest that the protein component of the diet is necessary for stimulating translation initiation in skeletal muscle during exercise recovery. The role of amino acids in stimulating muscle protein synthesis requires further study.

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REFERENCES


