Exercise Effects on Muscle Insulin Signaling and Action

Invited Review: Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise

SCOT R. KIMBALL,1 PETER A. FARRELL,2 AND LEONARD S. JEFFERSON1

1Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey 17033; and 2Noll Physiology Research Center, The Pennsylvania State University, University Park, Pennsylvania 16802

Kimball, Scot R., Peter A. Farrell, and Leonard S. Jefferson. Invited Review: Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. J Appl Physiol 93: 1168–1180, 2002. First published June 7, 2002; 10.1152/japplphysiol.00221.2002.—Protein synthesis in skeletal muscle is modulated in response to a variety of stimuli. Two stimuli receiving a great deal of recent attention are increased amino acid availability and exercise. Both of these effectors stimulate protein synthesis in part through activation of translation initiation. However, the full response of translation initiation and protein synthesis to either effector is not observed in the absence of a minimal concentration of insulin. The combination of insulin and either increased amino acid availability or endurance exercise stimulates translation initiation and protein synthesis in part through activation of the ribosomal protein S6 protein kinase S6K1 as well as through enhanced association of eukaryotic initiation factor eIF4G with eIF4E, an event that promotes binding of mRNA to the ribosome. In contrast, insulin in combination with resistance exercise stimulates translation initiation and protein synthesis through enhanced activity of a guanine nucleotide exchange protein referred to as eIF2B. In both cases, the amount of insulin required for the effects is low, and a concentration of the hormone that approximates that observed in fasting animals is sufficient for maximal stimulation. This review summarizes the results of a number of recent studies that have helped to establish our present understanding of the interactions of insulin, amino acids, and exercise in the regulation of protein synthesis in skeletal muscle.

Address for reprint requests and other correspondence: L. S. Jefferson, Dept. of Cellular and Molecular Physiology, The Pennsylvania State Univ. College of Medicine, 500 Univ. Drive, Hershey, PA 17033 (E-mail: jjefferson@psu.edu).

Protein synthesis in skeletal muscle is stimulated by either increased availability of amino acids, particularly the branched-chain amino acid leucine, or by resistance exercise. The protein synthetic response to both effectors is due in part to stimulation of the initiation of mRNA translation. Moreover, the response to either effector requires insulin-induced signaling to achieve a maximal effect on translation initiation and protein synthesis. Herein, we review the interactions of amino acids, exercise, and insulin in mediating translational control of protein synthesis in skeletal muscle. In regard to exercise, particular emphasis is placed on rodent models of resistance and endurance exercise.
TRANSLATIONAL CONTROL OF PROTEIN SYNTHESIS

Translation of mRNA is recognized as an important point of control of gene expression under a variety of circumstances. Indeed, mechanisms exist for discriminating which mRNAs are translated and to what extent. In general, these mechanisms provide for regulation of the initiation of mRNA translation, i.e., the assembly of a translationally competent ribosome at the AUG start site near the 5'-end of the mRNA (reviewed in Ref. 56). Translation initiation can be functionally divided into three stages: the binding of initiator methionyl-tRNA \((\text{met-tRNA}_i)\) to the 40S ribosomal subunit to form the 43S preinitiation complex, the binding of mRNA to the 43S preinitiation complex to form the 48S preinitiation complex, and the binding of the 60S ribosomal subunit to the 48S preinitiation complex to form the active 80S initiation complex (Fig. 1). At the first stage, met-tRNA\(_i\) binds to the 40S ribosomal subunit as a ternary complex consisting of eukaryotic initiation factor (eIF) eIF2, GTP, and met-tRNA\(_i\). Later in the process, the GTP bound to eIF2 is hydrolyzed and the eIF2-GDP binary complex is released from the initiation complex. Before eIF2 can bind another molecule of met-tRNA\(_i\), the GDP bound to eIF2 must be exchanged for GTP, a process catalyzed by another initiation factor, eIF2B. One mechanism for regulating the guanine nucleotide exchange activity of eIF2B involves phosphorylation of the \(\alpha\)-subunit of eIF2 on Ser\(^{51}\), whereby phosphorylation converts eIF2 from a substrate into a competitive inhibitor of eIF2B.

![Fig. 1. Translation initiation. The figure illustrates the stages in translation initiation involving the binding of methionyl-tRNA\(_i\) (met) and mRNA to the 40S ribosomal subunit. The translation initiation factors that participate in the individual stages are depicted as different geometric shapes and are labeled with a number or number and letter on the basis of the identity of the factor. For example, eukaryotic initiation factor (eIF) eIF4E is depicted as a hexagon with the label 4E in the center of the shape. In addition, the regulation of eIF2B activity by phosphorylation of the \(\alpha\)-subunit of eIF2 and the sequestration of eIF4E by eIF4E binding protein-1 (4E-BP1) are illustrated. mTOR, mammalian target of rapamycin; AUG, the nucleoside triplet adenosine, uridine, guanosine; GCN2, general control nonderepressing eIF2\(\alpha\) protein kinase 2. See text for further details.](http://jap.physiology.org/DownloadedFrom/10.220.33.4)
A second mechanism involves phosphorylation of Ser535 of the eIF2B, a modification that reduces the guanine nucleotide exchange activity of the protein.

At the second stage in initiation, eIF4F binds to mRNA, and the eIF4F-mRNA complex then binds to the 43S preinitiation complex. eIF4F is a heterotrimERIC complex consisting of eIF4A, an RNA helicase; eIF4E, the protein that binds to the m7GTP cap at the 5' end of the mRNA; and eIF4G, a scaffolding protein that, in addition to binding eIF4A and eIF4E, binds to eIF3 and the poly(A) binding protein. Assembly of eIF4A, eIF4E, and eIF4G into the eIF4F complex is regulated in part by the reversible association of eIF4E with eIF4E binding proteins such as eIF4E binding protein-1 (4E-BP1). The binding site on eIF4E for 4E-BP1 overlaps that for eIF4G. Thus binding of 4E-BP1 to eIF4E precludes the association of eIF4E with eIF4G and prevents assembly of the eIF4F complex. Formation of the 4E-BP1-eIF4E complex is regulated by phosphorylation of 4E-BP1, whereby hyperphosphorylation of the protein prevents its binding to eIF4E.

CONTROL OF TRANSLATION INITIATION VIA A SIGNAL-TRANSDUCTION PATHWAY INVOLVING THE MAMMALIAN TARGET OF RAPAMYCIN

Translation initiation is regulated in part by a signal-transduction pathway containing the serine/threonine protein kinase referred to as the mammalian target of rapamycin (mTOR, also known as FRAP or RAFT, reviewed in Ref. 63). Growth-promoting hormones such as insulin or insulin-like growth factor I (IGF-I) regulate mTOR through the sequential activation of a series of upstream kinases (Fig. 2A) including phosphatidylinositol-3 (PI-3) kinase and protein kinase B (PKB, also known as Akt, 65). PKB phosphorylates Ser2448 of mTOR in vitro (65), and insulin induces phosphorylation of this residue in cells in culture (55). Phosphorylation of Ser2448 in response to insulin is blocked by either amino acid deprivation or by treatment with the PI-3 kinase inhibitor wortmannin. Therefore, phosphorylation of mTOR on Ser2448 represents a potential point of integration of signals arising from insulin action with those generated by amino acid availability.

One downstream target of mTOR-mediated signaling is the translational repressor 4E-BP1. Results presented by Gingras et al. (38) suggest that phosphorylation of Thr37 and Thr46 by 4E-BP1 by mTOR acts as a priming event that allows the subsequent phosphorylation of Ser65 and Thr70 by an as yet unidentified protein kinase(s). Phosphorylation at Ser65 and Thr70 then causes dissociation of the 4E-BP1-eIF4E complex, allowing eIF4E to bind to eIF4G and form the active eIF4F complex. Further support for the suggestion that mTOR is acting as a priming kinase is provided by a more recent study showing that 4E-BP1 is phosphorylated in a hierarchical manner beginning with Thr37 and Thr46 (39). A caveat to this suggestion, however, is that, in contrast to Ser65 and Thr70, little change in phosphorylation of Thr37 or Thr46 is observed in cells treated with rapamycin (39), a specific inhibitor of mTOR, suggesting that the more COOH-terminal sites may be the direct targets of mTOR. However, whether or not amino acids directly promote activation of mTOR is unknown.

Another downstream target of signaling through mTOR is the ribosomal protein (rp) S6 protein kinase (S6K1, Fig. 2B, reviewed in Refs. 27, 36). Hyperphosphorylation of S6K1 results in its activation, which leads to hyperphosphorylation of the rp S6. Hyperphosphorylation of S6 is associated with enhanced translation of a particular class of mRNAs that contain a 5'-terminal oligopyrimidine structure. Proteins encoded by such mRNAs include the rps and translation elongation factors; i.e., proteins involved in mRNA translation. Thus activation of S6K1 ultimately results in enhanced translation.
in an increase in the capacity of the cell to synthesize protein. mTOR phosphorylates Thr\textsuperscript{389} on S6K1 in vitro assays, a site whose phosphorylation is critical for maximal activation of the kinase (19). Whether or not mTOR phosphorylates Thr\textsuperscript{389} in vivo is unknown. A second amino acid residue whose phosphorylation is essential for activation of S6K1 is Thr\textsuperscript{229}. This residue is phosphorylated by the phosphoinositide-dependent protein kinase, which is downstream of PI-3 kinase in the insulin signaling pathway (61). Thus hyperphosphorylation of S6K1 is regulated through the PI-3 kinase/mTOR signal-transduction pathway. However, S6K1 has numerous other phosphorylation sites, particularly those in the COOH terminus of the protein, that may act as priming sites in regulating the phosphorylation of Thr\textsuperscript{229} and Thr\textsuperscript{389}. Phosphorylation of the COOH-terminal sites is blocked by rapamycin (reviewed in Ref. 36), implicating mTOR in their regulation. It is noteworthy that four of the five COOH-terminal phosphorylation sites are followed by proline, as are the residues in 4E-BP1 that are phosphorylated in response to mTOR-mediated signaling. Thus it may be that mTOR-mediated signaling leads to phosphorylation of the C-terminal residues in S6K1, which then permit phosphorylation of Thr\textsuperscript{229} and Thr\textsuperscript{389}. Overall, it appears that activation of mTOR by insulin results in phosphorylation of residues on both 4E-BP1 and S6K1 that act as priming sites and permit subsequent phosphorylation by other regulatory kinases.

**ROLE OF AMINO ACIDS IN THE TRANSLATIONAL CONTROL OF PROTEIN SYNTHESIS IN SKELETAL MUSCLE**

Evidence for amino acids playing a role in the translational control of protein synthesis in skeletal muscle is provided by studies designed to investigate the effects of food intake in fasted animals. In skeletal muscle of fasted rats, protein synthesis is repressed compared with the fed state, and this is due in part to reduced mTOR-mediated signaling. For example, in the fasted state, 4E-BP1 in skeletal muscle is present predominantly in hypophosphorylated forms (25, 83, 84). Moreover, in fasted compared with freely fed animals, the amount of 4E-BP1 bound to eIF4E is elevated. Feeding a protein-containing meal, but not one lacking protein, rapidly restores protein synthesis to control (i.e., freely fed) values and results in hyperphosphorylation of 4E-BP1 and a decrease in the association of 4E-BP1 with eIF4E. In contrast, the amount of eIF4G bound to eIF4E is low in muscle of fasted animals and becomes elevated in response to feeding, indicating that feeding promotes assembly of the active eIF4F complex. The effect of feeding a meal containing protein on 4E-BP1 phosphorylation and assembly of eIF4F can be reproduced by oral administration of leucine alone (2). Indeed, oral leucine administration restores muscle protein synthesis to values observed in freely fed animals.

In addition to enhancing eIF4F assembly, either feeding a complete meal or oral administration of leucine also promotes hyperphosphorylation of S6K1 (2, 8, 46). Moreover, both phosphorylation of S6K1 and phosphorylation of rp S6 are increased within 15 min of oral leucine administration (4). However, phosphorylation of S6K1 returns to basal values within 2 h of leucine administration whereas rp S6 phosphorylation remains elevated. This result suggests that rp S6 phosphorylation is regulated through mechanisms in addition to changes in S6K1 activity.

The importance of changes in eIF4F assembly and hyperphosphorylation of S6K1 in the feeding-induced stimulation of muscle protein synthesis is demonstrated in studies using rapamycin to inhibit mTOR. In fasted rats orally administered leucine (5) or in fasted neonatal pigs fed porcine milk (46), prior treatment with rapamycin completely prevents hyperphosphorylation of 4E-BP1 and S6K1 as well as release of eIF4E from the inactive 4E-BP1-eIF4E complex. Rapamycin treatment also severely attenuates, but does not completely prevent, assembly of the eIF4F complex or the feeding-induced increase in muscle protein synthesis. These results suggest that enhanced eIF4F assembly and activation of S6K1 are critical components in the stimulation of muscle protein synthesis by feeding. However, it is clear that hyperphosphorylation of 4E-BP1 and S6K1 alone do not account entirely for the increase in protein synthesis. Indeed, signaling through an mTOR-independent pathway to promote assembly of the eIF4F complex is an additional mechanism involved in the stimulation of protein synthesis by amino acids. Evidence for the mTOR-independent pathway is provided in part by a study by Vary et al. (73) showing that increased provision of amino acids stimulates muscle protein synthesis in isolated rat hindlimb preparations perfused in the absence of insulin. In this experimental model, amino acids promote binding of eIF4G to eIF4E without affecting phosphorylation of either 4E-BP1 or S6K1 and with no change in 4E-BP1 binding to eIF4E. Thus amino acids regulate muscle protein synthesis through both an insulin- and mTOR-dependent signaling pathway, which results in phosphorylation of 4E-BP1 and S6K1, as well as an insulin- and mTOR-independent pathway, which results in enhanced assembly of eIF4F.

**REQUIREMENT FOR INSULIN-INDUCED SIGNALING IN THE ACTIVATION OF TRANSLATION INITIATION BY AMINO ACIDS**

In addition to increasing plasma amino acid concentrations, feeding a complete meal or oral administration of leucine also results in a rise in the circulating insulin concentration. Moreover, recent studies suggest that insulin and amino acids may act synergistically to enhance mRNA translation. For example, in human skeletal muscle, hyperphosphorylation of S6K1 is enhanced in response to increased plasma concentrations of either leucine or insulin, but a combination of the two effectors causes an even greater stimulation than either alone (42). Therefore, to define the role of insulin in the feeding-induced stimulation of muscle
protein synthesis, a variety of studies has attempted to prevent or attenuate the changes in plasma concentrations of the hormone that occur in response to feeding. Such studies can be divided into four categories on the basis of the approach used to prevent changes in plasma insulin concentration: injection of anti-insulin antibodies, diazoxide administration, somatostatin infusion, and use of diabetic animals. The following paragraphs summarize results obtained by using each of these approaches.

The approach of using anti-insulin antibodies has the advantage of inducing a rapid fall in the plasma insulin concentration, thus avoiding complicating effects generated by agents used to induce diabetes or secondary effects resulting from long-term insulin deprivation. The results of such studies (52, 60, 69) consistently show that the plasma insulin concentration is important for maximal stimulation of muscle protein synthesis by amino acids. In one study, anti-insulin antibodies blocked completely the amino acid-induced stimulation of muscle protein synthesis (60), whereas in two other studies they attenuated, but did not completely prevent, the stimulation (52, 69).

A second approach to defining the role of insulin in the feeding-induced stimulation of protein synthesis in skeletal muscle is the use of diazoxide, which inhibits secretion of the hormone from pancreatic beta cells. Sinaud et al. (67) show that protein synthesis in soleus, gastrocnemius, and epitrochlearis muscles is reduced by diazoxide treatment, suggesting that insulin is required for the stimulation of protein synthesis independent of fiber type. Diazoxide treatment also reduces the amount of eIF4G bound to eIF4E and enhances the association of eIF4E with 4E-BP1, suggesting that insulin deprivation inhibits muscle protein synthesis by reducing eIF4F assembly. In a second study (8), they show that diazoxide treatment maintains plasma insulin concentrations at fasted values after ingestion of either a protein-containing or a protein-free meal. Feeding a protein-containing meal, but not a protein-free meal, stimulates protein synthesis as measured in vitro in isolated epitrochlearis muscle. Diazoxide treatment represses protein synthesis below the value observed in fasted animals independent of the meal administered. Feeding the protein-containing meal, but not the protein-free meal, enhances phosphorylation of S6K1 and 4E-BP1, reduces binding of 4E-BP1 to eIF4E, and enhances assembly of the eIF4F-eIF4G complex. The effect of feeding a protein-containing meal on changes in 4E-BP1 phosphorylation and binding of 4E-BP1 to eIF4E is abrogated by diazoxide treatment. Moreover, in diazoxide-treated animals, eIF4G binding to eIF4E is reduced below fasted values. Interestingly, in animals fed a protein-free meal, diazoxide treatment increases the binding of 4E-BP1 to eIF4E above the fasted value and reduces 4E-BP1 phosphorylation and eIF4G binding to eIF4E to below detectable levels. Overall, the results of their studies suggest that an increase in plasma insulin is required for the stimulation of protein synthesis in response to feeding a protein-containing meal. Moreover, increased assembly of the eIF4F complex is associated with the stimulation of protein synthesis induced by feeding a protein-containing meal.

A third approach to assessing the contribution of insulin to the feeding-induced stimulation of muscle protein synthesis is the use of somatostatin to inhibit insulin secretion from pancreatic beta cells. In a study by Anthony et al. (3), a significant rise in the plasma insulin concentration is observed within 15 min of oral leucine administration even though serum and intramuscular leucine concentrations remain elevated for at least 2 h. 4E-BP1, S6K1, and S6 hyperphosphorylation and eIF4G binding to eIF4E are increased whereas 4E-BP1 binding to eIF4E is decreased by 15 min and maximally changed by 30 min of leucine administration. However, the increase in protein synthesis in gastrocnemius muscle is not observed until 30 min after administration. In contrast to the response of the plasma insulin concentration, changes in protein synthesis are maintained for at least 1 h but return to baseline by the 2-h time point. Thus protein synthesis returns to control values even though serum and intramuscular leucine concentrations remain elevated, suggesting that both increased leucine and insulin are required for sustained stimulation. Somatostatin prevents the leucine-induced changes in plasma insulin and protein synthesis. However, somatostatin does not prevent leucine-induced changes in eIF4E binding to 4E-BP1 or eIF4G, suggesting that such changes are not sufficient by themselves to stimulate protein synthesis. Moreover, somatostatin attenuates the leucine-induced hyperphosphorylation of 4E-BP1 and S6K1 and completely prevents changes in phosphorylation of S6. Overall, the results of this study agree with those of Grizard and co-workers (8, 67) and suggest that the feeding- and leucine-induced increases in muscle protein synthesis above fasting values require an increase in the plasma insulin concentration. The different response of translation initiation factors observed between the two sets of studies may be due to differences in diet, i.e., a complete meal compared with leucine alone. However, it is more likely that the time at which the inhibitor of insulin secretion is administered relative to feeding accounts for the differences. Thus Anthony et al. (3) administered somatostatin before oral administration of leucine to prevent the leucine-induced increase in plasma insulin concentration. This approach is different from that used by Grizard and co-workers (8, 67) in which diazoxide is administered 1 h after feeding, which reduces the already elevated plasma insulin concentration rather than preventing it from rising during the first hour after feeding.

A fourth approach to assessing the contribution of insulin to the feeding-induced stimulation of muscle protein synthesis involves the use of rodent models of diabetes. Using ob/ob mice, an animal model of Type II diabetes, and nonobese diabetic mice, an animal model.
of Type I diabetes, Svanberg et al. (69) show that protein synthesis is increased in skeletal muscle by feeding in the absence of changes in the plasma insulin concentration. A subsequent study (68) extends the original one to show that, in both ob/ob and nonobese diabetic mice, feeding promotes hyperphosphorylation of 4E-BP1 and S6K1, as well as decreasing 4E-BP1 binding to eIF4E and enhancing association of eIF4E with eIF4G. However, these studies do not reveal the contributory role of insulin in the feeding-induced responses because, in both animal models, plasma concentrations of the hormone are elevated in fasted compared with control animals, and, in ob/ob mice, plasma insulin concentrations are increased by feeding.

In a study using alloxan to induce diabetes in rats, Anthony et al. (4) show that the fasting plasma insulin concentration is reduced to ~25% of the value observed in control animals and does not change after oral leucine administration. They further show that protein synthesis in gastrocnemius muscle of diabetic rats is ~25% of the value in control animals. Interestingly, protein synthesis is stimulated by oral leucine administration in both control and diabetic animals. However, the magnitude of the increase in diabetic rats is less than in control animals and remains below the value observed in fasted control animals even after leucine administration. Infusion of insulin into diabetic rats at rates sufficient to restore plasma concentrations of the hormone to those observed in fasted control rats (low insulin infusion) has no effect on protein synthesis unless leucine is also administered. In contrast, restoration of insulin to levels found in fed, control animals (high insulin infusion) stimulates protein synthesis to values observed in fasted, control animals, and leucine administration has no further stimulatory effect under such conditions. In diabetic animals, leucine administration has no effect on phosphorylation of 4E-BP1, S6K1, or S6, or eIF4E association with 4E-BP1 or eIF4G. Low insulin infusion alone has no effect on any of these parameters, but in combination with leucine administration it causes increased phosphorylation of 4E-BP1 and S6K1, decreased binding of 4E-BP1 to eIF4E, and enhanced assembly of the eIF4G-eIF4E complex. High insulin infusion alone results in changes similar to those observed in diabetic animals subjected to low insulin infusion and also administered leucine. Leucine administration to high-insulin-infusion animals causes additional changes in all parameters and in particular results in dramatic increases in phosphorylation of S6K1 and S6.

Overall, the results presented by Anthony et al. (4) suggest that at a subphysiological plasma insulin concentration (i.e., that observed in the diabetic rats used in these studies), leucine enhances protein synthesis through an mTOR-independent signaling pathway that does not involve activation of S6K1 or increased assembly of eIF4F. However, activation of the mTOR-independent signaling pathway by leucine does not elevate protein synthesis to the control value unless the plasma insulin concentration is restored to the control value. Thus, at physiological plasma insulin concentrations, leucine activates both mTOR-dependent and -independent signaling pathways that act together to optimally stimulate protein synthesis.

Further support for an interactive role for insulin and amino acids in stimulating translation initiation derives from in vitro studies using cells in culture. For example, insulin and amino acids independently promote phosphorylation of 4E-BP1 in primary cultures of rat adipocytes but are more effective when provided in combination (57). Moreover, addition of either insulin or amino acids increases S6K1 activity ~50% in Chinese hamster ovary cells overexpressing the insulin receptor, whereas together they cause a greater than fivefold increase in activity (22). Thus, as is the case for the in vivo studies described above, in cells in culture, amino acids and insulin interact to promote various individual steps in translation initiation.

**ROLE OF EXERCISE IN THE TRANSLATIONAL CONTROL OF PROTEIN SYNTHESIS IN SKELETAL MUSCLE**

The response of protein synthesis in skeletal muscle to exercise varies with the type of exercise and with the time of measurement. For example, most, but not all (72), studies show that protein synthesis declines or does not change during exercise (24, 26). In contrast, synthesis of mixed muscle proteins (23, 29, 34, 35, 58, 80, 82), myofibrillar proteins (77, 80), and myosin heavy chain proteins (9) increases after resistance exercise.

Early studies that examined the mechanism through which protein synthesis is attenuated during exercise suggest that all three steps in mRNA translation, i.e., initiation, elongation, and termination, might be affected. For example, results from a study by Bylund-Fellenius et al. (20) demonstrate that the reduction in protein synthesis that occurs during electrically induced muscle contractions is probably due to a change in all three steps of translation. A reduction in the energy status of the muscle, i.e., reduced ATP and creatine phosphate content, decreased ATP-to-creatine phosphate ratio, and elevated lactate, closely mirrors the fall in protein synthesis suggesting that during exercise, as opposed to during the recovery period, energy status is an important determinant of protein synthetic rates. Results from another study that examined changes occurring during the recovery period after exercise (7) show that translation initiation is enhanced in extensor digitorum longus muscle undergoing high-intensity lengthening contractions. In contrast, no change in translation initiation is observed in the same leg in soleus muscle undergoing primarily concentric contractions. The same study shows that changes occurring during the recovery period from electrical stimulation are most prominent 6 h after exercise. The importance of the type of contraction, eccentric vs. concentric, requires clarification because several reports (58, 59, 80, 81) suggest that both types of contractions cause increases in protein synthesis.
A consistent finding, despite markedly different experimental models of exercise, is that muscle protein synthesis is elevated after acute resistance exercise with no change in muscle RNA content (23, 29, 34, 35, 49, 50, 75–77, 80, 81). In fact, Caiozzo et al. (21) report that 16 days of in situ stimulation of medial gastrocnemius are required to cause changes in total RNA. In the model used in those studies, muscle contractions occur against synergist muscles only. Thus the changes in protein synthesis that occur in the first hours to days after resistance exercise are not due to an increase in muscle ribosome content (as indicated by no change in RNA) but instead are related to enhanced mRNA translation. Moreover, while not excluding regulation of translation elongation or termination, the reports cited above suggest that mechanisms that control increases in protein synthesis after acute resistance exercise may reside in the process of translation initiation. Thus early changes in mRNA translation (i.e., those occurring within the first few minutes to hours after exercise) are a result of increased translation of existing mRNAs, whereas the response at later times (i.e., hours to days after exercise) is enhanced by increased ribosome number.

The first report to demonstrate a role for translation initiation in the hypertrophic response of muscle overload is that of Augert et al. (6). That study utilizes a model of soleus hypertrophy induced by 4 days of gastrocnemius ablation. The results of the study show that in soleus muscle treated with pactamycin to inhibit the formation of new peptide chains without preventing the elongation of chains already initiated before treatment with the drug, incorporation of radiolabeled leucine into protein is higher in overloaded muscles compared with controls, suggesting that translation initiation is elevated during overload conditions. The study also includes results obtained using gold-thioglucose (GTG) to induce hyperinsulinemia. A comparison of the results obtained when using these animals shows that ribosomal transit times, a measure of peptide-chain elongation, are not altered in hypertrophied muscle from either control or GTG-treated mice. Because of what the authors refer to as insulin resistance, the increase in muscle mass and leucine incorporation into hypertrophying muscle is reduced in GTG-treated mice. However, although the rate of protein synthesis is reduced in GTG-treated mice, muscle mass is still increased to about half the degree as control mice. Overall, the findings of the report by Augert et al. suggest that translation initiation is a point of regulation for protein synthesis in this particular model of muscle hypertrophy.

The first study to describe the role of specific translation initiation factors in the response of protein synthesis in skeletal muscle to endurance exercise is that of Gautsch et al. (37). The results of that study indicate that protein synthesis in skeletal muscle is depressed shortly after prolonged treadmill running and that its recovery is augmented by provision of a complete meal but not by one lacking protein. Moreover, the study demonstrates that assembly of the eIF4F complex is associated with restoration of protein synthesis by meal feeding after endurance exercise.

In addition to assembly of the eIF4F complex, translation initiation is also regulated by changes in the guanine nucleotide exchange activity of eIF2B (Fig. 3). For example, a reduction in the activity of eIF2B in skeletal muscle is associated with a decrease in protein synthesis in severely diabetic rats (44, 45, 47) but not moderately diabetic rats (29). Although the mechanism by which insulin regulates eIF2B activity in vivo is as yet undefined, in cells in culture the hormone promotes dephosphorylation of Ser535 on the ε-subunit of the protein, an event that correlates with increased guanine nucleotide exchange activity in cell extracts (78, 79). Regulation of Ser535 phosphorylation by insulin is mediated by a signaling pathway involving PKB and glycogen synthase kinase (GSK)-3. In contrast to the fall in eIF2B activity in muscle of diabetic animals, the increase in muscle protein synthesis that occurs 16 h after acute resistance exercise is associated with an elevation in eIF2B activity (29). Under such conditions, rates of protein synthesis and eIF2B activity are increased in both nondiabetic and moderately diabetic rats but not in severely diabetic rats (48). The mechanism through which eIF2B activity is upregulated by resistance exercise is unknown but has been shown not to be related to exercise-induced changes in plasma insulin, corticosterone, or total muscle RNA. Whether or not resistance exercise modulates phosphorylation of eIF20 or eIF2Be is presently unknown.

As described above, the results of the study by Gautsch et al. (37) suggest that increased eIF4F assembly is

![Diagram of translation initiation factors](http://jap.pathology.org/)

**Fig. 3. Inhibition of eIF2B by glycogen synthase kinase (GSK)-3.** GSK-3 phosphorylates Ser535 on the ε-subunit of eIF2B, resulting in a reduction in guanine nucleotide exchange activity. Insulin inhibits GSK-3 through a signal-transduction pathway involving PI-3 kinase and protein kinase B (PKB). Exercise stimulates the guanine nucleotide exchange activity of eIF2B in extracts of gastrocnemius muscle. One potential mechanism through which exercise might enhance eIF2B activity, dephosphorylation of eIF2Be, is illustrated in the figure. Although evidence for changes in eIF2Be phosphorylation in response to exercise has not been reported, it is clear that exercise enhances eIF2B activity in control and moderately diabetic rats, providing support for such an idea.
important for restoring rates of protein synthesis to basal values after endurance exercise. However, changes in eIF4F assembly may not be involved in the stimulation of muscle protein synthesis after resistance exercise. For example, a study by Farrell et al. (30) shows that the increase in protein synthesis observed 16 h after resistance exercise is not accompanied by changes in eIF4E binding to 4E-BP1 or eIF4G. Moreover, 4E-BP1 and eIF4E phosphorylation is not significantly affected by prior exercise. However, it should be noted that the study shows a trend \( P = 0.09 \) toward an increase in the amount of eIF4E associated with eIF4G. This observation may be important because recent data show that eIF4E-eIF4G association is regulated by mechanisms in addition to changes in eIF4E binding to 4E-BP1 (74). The latter study, in combination with the studies demonstrating changes in eIF2B activity after resistance exercise, supports the working hypothesis that eIF2B activity has a greater influence on mRNA translation after acute resistance exercise compared with modulation of eIF4F assembly.

In contrast to results obtained when using acutely exercised animals, a recent study by Bodine et al. (17) suggests that eIF4F assembly may play an important role in regulating muscle protein synthesis after overload-induced hypertrophy. In that study, 14 days after functional overload of the plantaris by synergist ablation, the amount of PKB and its phosphorylation status is markedly increased, as is phosphorylation of 4E-BP1. Enhanced assembly of the eIF4F complex, reduced binding of 4E-BP1 to eIF4E, and hyperphosphorylation of S6K1 are also reported. In vivo treatment with rapamycin to block mTOR-mediated signaling reverses the observed changes in phosphorylation of 4E-BP1 and S6K1 during overload and severely blunts hypertrophy of the plantaris. Rapamycin given to control rats has no effect on muscle weight, demonstrating that pathways involved in the adaptation to overload are not necessary for maintaining basal muscle mass. The study also includes a model of disuse, i.e., hindlimb suspension. In the experimental model of disuse, 14 days of unloading causes decreased phosphorylation of PKB and S6K1 and an increase in 4E-BP1-eIF4E complex assembly. The importance of the PKB/mTOR signaling pathway to muscle hypertrophy is further demonstrated by overexpression of a constitutively active form of PKB by plasmid DNA injection. Such treatment causes muscle hypertrophy in resting adult mice and blunts the atrophy associated with disuse. Furthermore, the changes are reversed by treatment with rapamycin. A recent study by Reynolds et al. (64) demonstrates that phosphorylation of Ser2448 of mTOR by PKB is an important regulator for hypertrophy of plantaris muscle using the same overload model as described by Bodine et al. (17) and provides further support for a role of mTOR in mediating the observed changes in muscle mass. Overall, the reports by Bodine et al. and Reynolds et al. (64) clearly demonstrate that the PKB/mTOR signaling pathway that regulates eIF4F assembly and S6K1 activation is critical to overload-induced or genetically manipulated hypertrophy. However, the reports do not contain any information on the effects of the various manipulations on plasma concentrations of insulin and, therefore, conclusions cannot be drawn about the contributing role of the hormone in the various responses. Related to this point, Hernandez et al. (43) found no differences in arterial insulin concentrations for 24 h after acute resistance exercise.

A potential explanation for the discrepancy between acute resistance exercise, which does not seem to alter 4E-BP1 phosphorylation or 4E-BP1-eIF4E complex stability (30), and constant overload, which does (17), is that, by necessity, overloaded muscles are studied while the overload is being applied. In contrast, the resistance exercise studies described earlier are performed 16 h postexercise, a period when rates of protein synthesis are found to be elevated. Thus changes in eIF4F assembly may increase for a few hours after acute resistance exercise but return to basal values later in the recovery period. Further work is required to address this apparent discrepancy.

In contrast to the experimental model of overloaded muscle used by Bodine et al. (17), Markuns et al. (51) show that when animals are studied immediately and at 5, 10, 30, or 60 min after moderate intensity treadmill running, neither PKB activity nor phosphorylation of PKB on Ser\(^{473} \) is changed. Sherwood et al. (66) and Brozinick and Birnbaum (18) report similar results when using alternative experimental models of muscle contractions in which PKB is unaffected by contractions or exercise. Although PKB activity does not change in these experimental models, the available evidence suggests that the activity of GSK-3 is regulated by contractions. For example, GSK3\( \beta \) phosphorylation (which leads to enzyme inactivation) is increased in one study (17), and the activity of both GSK3\( \alpha \) and GSK3\( \beta \) is reduced by exercise in a second study (51). Finally, Nader and Esser (53) show that different forms of exercise alter extracellular-regulated protein kinase, PKB, S6K1, and p38 phosphorylation and that different time courses for such changes are observed in anterior tibialis compared with soleus muscle. Moreover, S6K1 phosphorylation remains elevated for 3–6 h after high-frequency electrical stimulation, and PKB activity is higher immediately after stimulation but returns to control values by 3 h poststimulation. These data are consistent with a previous study that used acute resistance exercise to show that the activity of S6K1 is elevated 6–24 h after acute resistance exercise but not 0–6 h immediately after exercise (43).

An extensive discussion of how different types of muscle contraction affect translational control is not possible at this time given the present state of knowledge in this area. It is, however, necessary to note the wide differences in models used in the studies reported in the available literature. Pertinent characteristics of the models and findings relative to mRNA translation are compiled in Table 1. The Farrell (28–30, 48), Bodine (17), and Augert (6) groups used models that result in muscle hypertrophy when exercise (28) or overload...
Table 1. Characteristics of the models used to date to study components of mRNA translation

<table>
<thead>
<tr>
<th>Reference (Rats or Mice)</th>
<th>Type of Contract and Number of Sessions</th>
<th>Hours After Last Exercise Session When Muscles Were Excised</th>
<th>Phenotypic Muscle Response to Chronic Stimulus</th>
<th>eIF2B</th>
<th>4E-BP1 Associated with eIF4E</th>
<th>eIF4G Associated with eIF4E</th>
<th>S6K1</th>
<th>mTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farrell et al. (30) nondiab moderate diabetes</td>
<td>Ecc/con Acute Resistance 4 sessions</td>
<td>16 Gastrocnemius</td>
<td>Hypertrophy when performed for 6 wks (28)</td>
<td>↑</td>
<td>→</td>
<td>→</td>
<td>↑</td>
<td>?</td>
</tr>
<tr>
<td>Kostyak et al. (48) severe diabetes</td>
<td>Ecc/con Acute Resistance 4 sessions</td>
<td>16 Gastrocnemius</td>
<td>Change in muscle mass would be doubtful</td>
<td>→</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Bodine et al. (17) nondiab</td>
<td>Ecc/con Overload 14 d Continuous Stimulus</td>
<td>0 Plantaris</td>
<td>Hypertrophy</td>
<td>?</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Bodine et al. (17) nondiab</td>
<td>Inactivity by Hindlimb Suspension or Denervation 14 d</td>
<td>0 Tibialis anterior</td>
<td>Atrophy</td>
<td>?</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Augert et al. (6) nondiabetic and hyper-insulinemic</td>
<td>Ecc/con Overload 4 d</td>
<td>0 Soleus</td>
<td>Hypertrophy (increased translation initiation with no change in elongation)</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Gautsch et al. (37) nondiab</td>
<td>Ecc/con Running 1 session</td>
<td>1 Gastrocnemius/Plantaris</td>
<td>Oxidative adaptations</td>
<td>?</td>
<td>↓</td>
<td>↑</td>
<td>→</td>
<td>?</td>
</tr>
</tbody>
</table>

→, No change with exercise; ↓, decrease or ↑, increase with exercise; ?, not studied; ecc, eccentric contractions; eIF, eukaryotic initiation factor; 4E-BP1, eIF4E binding protein-1; S6K1, S6 protein kinase; mTOR, mammalian target of rapamycin; con, concentric contractions; nondiab = nondiabetic rats or mice.

(6, 17) are performed chronically. In contrast, treadmill running protocols such as that used by Gautsch et al. (37) lead to elevations in mitochondrial density, enzyme activity of both beta oxidation and tricarboxylic acid cycles, and the ability to deliver oxygen to the recruited muscle units, to name only a few adaptations. All of the models require both eccentric and concentric contractions; however, the intensity of each contraction varies from intense, as in the studies reported by Farrell and colleagues (28–30, 48), to less intense but repeated during every step the animals take, as in the studies reported by Augert et al. (6) and Bodine et al. (17). Such differences may result in varying degrees of muscle damage, amino acid availability for protein synthesis, differences in hormonal responses, and differences in blood flow, all of which could alter the ability to mount an anabolic response. The studies also differ in the muscles studied. Augert et al. (6) studied soleus muscle and reported elevations in rates of initiation of mRNA translation. The model used in the studies reported by Farrell and colleagues (28–30, 48) requires eccentric and concentric contractions of gastrocnemius and soleus (but not extensor digitorum longus), and in those studies elevations in rates of protein synthesis and eIF2B activity in gastrocnemius muscles were reported; soleus muscle was not studied. Bodine et al. (17) studied plantaris after synergist ablation and found increases in eIF4G-eIF4E complex formation and dissociation of 4E-BP1-eIF4E due to elevated phosphorylation of 4E-BP1. Thus muscles with predominantly type I (soleus) and type II (gastrocnemius and plantaris) fiber characteristics can hypertrophy, and adaptation shows a dependence on several important regulators of mRNA translation. Studies that examined S6K1 and mTOR in addition to components of translation are also included in Table 1 because they may be upstream regulators of the mRNA binding step in translation.

In summary, acute resistance exercise results in activation of the guanine nucleotide exchange activity of eIF2B. A stimulation of translation initiation and protein synthesis that occurs in skeletal muscle in response to endurance exercise is associated with hyperphosphorylation of 4E-BP1 but perhaps not S6K1 (37, 53). One model of resistance exercise shows no effect on eIF4E availability but does show an involvement of S6K1, whereas a model of continuous overload shows a dependence on both eIF4E availability and S6K1. “Exercise” is a very complex stimulus in terms of intracellular regulation. The pathways activated depend on characteristics of the contractions required to perform the movement, and the time course of such activation is far from clear.

**REQUIREMENT FOR INSULIN IN THE ACTIVATION OF TRANSLATION INITIATION BY EXERCISE**

Some studies (reviewed in Ref. 40) suggest that insulin is not necessary for the muscle hypertrophy that occurs in response to overload. For example, a report...
by Goldberg (41) shows that hypertrophy of soleus occurs in both legs of diabetic rats subjected to unilateral synergist ablation (41). However, in that report, the animals studied may have been only mildly diabetic, because plasma glucose averaged only 350 mg/dl. Furthermore, the report does not provide information on the plasma insulin concentration. The results of another study using moderately diabetic rats (29) are consistent with that of Goldberg in showing that protein synthesis increases after acute resistance exercise in moderately diabetic rats. Moreover, an increase in muscle mass occurs in moderately diabetic rats that perform chronic resistance exercise over many weeks (28). However, in severely diabetic rats, no change in muscle protein synthesis is observed in response to acute resistance exercise (28). Taken together, the results of the studies summarized here suggest that a certain concentration of plasma insulin (i.e., that present in a mildly diabetic rat) is required for both increased protein synthesis and accretion of muscle mass in response to resistance exercise.

Further evidence supporting the concept that a critical concentration of plasma insulin is needed for the stimulation of protein synthesis that occurs after resistance exercise is provided by the report of Fluckey et al. (34). In the model used by these investigators, rats either are exercised or remain sedentary and are subsequently subjected to bilateral hindlimb perfusion in which one leg is perfused with medium containing insulin and the other with medium devoid of the hormone. The perfusion medium additionally contains physiological concentrations of amino acids. In the presence of insulin, protein synthesis is stimulated in the perfused muscle preparations from exercised compared with those from sedentary rats. In contrast, the exercise-induced stimulation of protein synthesis is not observed when insulin is eliminated from the perfusion medium. These findings show conclusively that insulin, in combination with prior contractions, induces a stimulation of protein synthesis.

The studies described above suggest that insulin is required for the exercise-induced stimulation of protein synthesis in skeletal muscle. However, results obtained when using moderately diabetic rats imply that the insulin concentration necessary for the effect may be minimal. To address the question of what concentration is required for the permissive effect of insulin on protein synthesis, Fedele et al. (31) compared muscle protein synthesis to plasma insulin concentration in animals exhibiting a wide range of plasma concentrations of the hormone. The results of that study, illustrated diagrammatically in Fig. 4, show that rates of protein synthesis are related to arterial insulin only in the very low range (<80 pmol/l for a 5-h fasted rat). Below this threshold concentration, muscle protein synthesis is low and cannot be elevated by resistance exercise. Such low insulin concentrations correspond to glucose concentrations around 550 mg/dl in the 5-h fasted state. In contrast, protein synthesis becomes independent of increases in plasma insulin when the concentration increases above ~100 pM. Under such conditions, contraction leads to a stimulation of muscle protein synthesis.

The question arises whether there are compensatory factors that can assist in the stimulation of muscle protein synthesis during severe hypoinsulinemia. IGF-I is a likely candidate because 1) it can stimulate protein synthesis independent of insulin (11) and 2) in moderately diabetic, but not nondiabetic, rats, muscle IGF-I concentrations increase both after moderate resistance exercise and in the synergist ablation model (1). Furthermore, results from a study by Fedele et al. (32) show that passive immunization against IGF-I before, during, and after resistance exercise abolishes the exercise-induced stimulation of protein synthesis in moderately diabetic rats but has no effect in nondiabetic rats. Such a compensatory role for IGF-I is also evident in a fasting-refeeding model in which muscle protein synthesis is not increased by feeding in moderately diabetic rats that are passively immunized against IGF-I (33). In contrast, similar rats that receive nonspecific IgG infusions exhibit increased protein synthesis.

INTERACTION OF AMINO ACIDS, INSULIN, AND EXERCISE AND THE REGULATION OF PROTEIN SYNTHESIS IN SKELETAL MUSCLE

The results of studies using humans or animals have provided insights into the interrelationship among amino acid availability, resistance exercise, and insulin in the regulation of protein synthesis in skeletal muscle. Several lines of evidence suggest that amino acid availability is required for an anabolic response in protein metabolism to resistance exercise and that exercise itself enhances such availability. Infusion of essential but not nonessential amino acids after resistance exercise results in a change from a net negative protein balance to a significantly less negative protein
balance (16, 58, 59, 71). Meals taken immediately before (72) or after exercise are necessary for maintaining a less negative protein balance (16, 70) and for elevations in rates of protein synthesis (37, 43, 62). The availability of the ingested amino acids increases after exercise because of increased uptake of specific amino acids (14, 15, 54, 72, 85, 86), and this increase may be blood flow dependent (16). Leg blood flow is elevated after resistance exercise, consistent with the known vasodilatory actions of insulin (12, 13); however, infusion of insulin directly into the femoral artery increases blood flow at rest but no further augmentation is observed when insulin is infused after resistance exercise (14). Others (10) have reported similar results in rats; however, one study (24) suggests that prior exercise enhances the effect of insulin on protein synthesis. None of the reports cited in this paragraph provide information on the effects of the combination of amino acid availability, contractions, and insulin on translation initiation, and, therefore, no conclusions can be drawn about the interactions of the various interventions on specific initiation factors.

CONCLUSIONS

Overall, the preponderance of information presented herein supports a requirement for a minimal concentration of plasma insulin in either the amino acid- or exercise-induced stimulation of translation initiation and protein synthesis in skeletal muscle. In particular, insulin availability is important in mTOR-mediated signaling as evidenced by the hyperphosphorylation of 4E-BP1 and S6K1 and for maximal binding of eIF4G to eIF4E to form the active eIF4F complex that occurs in response to ingestion of a protein-containing meal or the oral administration of leucine. Such changes in mTOR signaling not only enhance synthesis of protein on a global scale but also would be expected to increase the capacity of the tissue to synthesize protein as a result of increased ribosome biogenesis. Stimulation of protein synthesis after acute resistance exercise is associated with enhanced activity of eIF2B. Initial reports using a muscle overload model show a dependence on eIF4E availability through mechanisms that include mTOR and PKB. Regulatory pathways in models of chronic continuous overload may differ from models of acute resistance exercise because eIF4E availability seems to be important in the former but not the latter. There appears to be a requirement for a minimal concentration of plasma insulin in generating a stimulation of muscle protein synthesis and eIF2B activity in response to resistance exercise. The amount of insulin required for the effect is low, with insulin concentrations equivalent to or less than fasting values being sufficient for the effect.

FUTURE DIRECTIONS

Only a few of the stages in translation initiation shown in Figs. 1 and 2 have been investigated from the perspective of either amino acid- or exercise-induced elevations in protein synthesis in skeletal muscle. Accelerated progress in our understanding of the mechanisms involved in mediating the effects of amino acids and exercise as well as those involved in the requirement for a minimal concentration of plasma insulin will require the use of a combination of whole body experimental models coupled with in vitro techniques. One particularly perplexing question involves how mTOR regulates amino acid-induced stimulation of 4E-BP1 and S6K1 phosphorylation. In particular, does amino acid-induced signaling modulate the protein kinase activity of mTOR, or does it function in parallel to the mTOR signaling pathway? Another unanswered question is how amino acid-induced signaling regulates assembly of the eIF4F complex. In part, amino acid-induced signaling promotes eIF4E binding to eIF4G through an mTOR-dependent process. However, it also appears to modulate eIF4F complex assembly through an mTOR-independent mechanism. Finally, identification of which kinases alter either eIF2α or eIF2β phosphorylation will be critical to identifying why eIF2B activity is elevated after acute resistance exercise. Genetic approaches may prove valuable in this regard; however, all attempts to construct an eIF2B knockout mouse or insect have proven fatal. Other strategies are obviously needed and warranted.

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