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Role of eIF4E in stimulation of protein synthesis by IGF-I in perfused rat skeletal muscle

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Vary, Thomas C., Leonard S. Jefferson, and Scot R. Kimball. Role of eIF4E in stimulation of protein synthesis by IGF-I in perfused rat skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 278: E58–E64, 2000.—Insulin-like growth factor I (IGF-I) promotes anabolism by stimulating protein synthesis in skeletal muscle. In the present study, we have examined mechanisms by which IGF-I stimulates protein synthesis in skeletal muscle with a perfused rat hindlimb preparation. IGF-I (10 nM) stimulated protein synthesis over 2.7-fold. Total RNA content was unaffected, but translational efficiency was increased by IGF-I. We next examined the effect of IGF-I on eukaryotic initiation factor (eIF) 4E as a mechanism regulating translation initiation. IGF-I did not alter either the amount of eIF4E associated with the eIF4E binding protein 4E-BP1 or the phosphorylation state of 4E-BP1. Likewise, the phosphorylation state of eIF4E was unaltered by IGF-I. In contrast, the amount of eIF4E bound to eIF4G was increased threefold by IGF-I. We conclude that IGF-I regulates protein synthesis in skeletal muscle by enhancing formation of the active eIF4E·eIF4G complex.

insulin-like growth factor I; eukaryotic initiation factors 4E and 4G; 4E binding protein 1

INSULIN-LIKE GROWTH FACTOR I (IGF-I) is a circulating hormone synthesized predominantly by the liver. It shares structural and functional similarities with insulin, and like insulin, IGF-I plays a distinct role in the regulation of whole body protein metabolism. Systemically administered, IGF-I results in weight gain in normal rats, reduces weight loss during starvation or diabetes (38), and attenuates protein loss during cachetic states (8, 47, 48).

Part of the anabolic action of IGF-I on protein metabolism is mediated through an increase in protein synthesis in muscle (2, 20, 43). Recombinant human IGF-I stimulates protein synthesis in freshly isolated myocytes from adult rats or in hearts perfused in vitro (16). In myotubes or myoblasts from the L8 or L6 cell lines in culture, IGF-I is a more potent stimulator of protein synthesis than insulin (1, 18). Likewise, IGF-I stimulates protein synthesis in incubated muscles (7, 43, 49) or perfused hindlimb (25). Indeed, Lang et al. (31) demonstrated a positive linear relationship between IGF-I and protein synthesis in skeletal muscle. In humans, intravenous infusion of IGF-I directly increases protein synthesis in skeletal muscle, provided that plasma amino acid concentrations are maintained (15, 42). Taken together, these observations indicate that IGF-I directly stimulates protein synthesis in skeletal muscle.

However, the mechanism by which IGF-I stimulates protein synthesis is presently unknown. Considerable evidence suggests that the binding of mRNA to the 43S preinitiation complex, which is mediated by eukaryotic initiation factor (eIF) 4F, can control the overall rate of protein synthesis. One of the subunits of the eIF4F complex, eIF4E, binds the 7-methylguanosine 5′-triphosphate (m^7GTP) cap structure present at the 5′-end of eukaryotic mRNAs to form an eIF4E·mRNA complex (40). During translation initiation, the eIF4E·mRNA complex binds to eIF4G and eIF4A to form the active eIF4F complex (40, 41, 45). The active eIF4E·eIF4G complex allows binding of mRNA to the 43S preinitiation complex and elongation then proceeds.

eIF4E is regulated by several mechanisms. First, phosphorylation of eIF4E enhances the affinity of the factor for m^7GTP cap analogs of mRNA (33) and for eIF4G and eIF4A (35). Increased phosphorylation of eIF4E correlates with enhanced rates of protein synthesis in cultures of cells stimulated with mitogens, growth factors, or serum (35–37) or transformed with ras or src oncogenes (11). Conversely, reduced phosphorylation of eIF4E correlates with an inhibition of protein synthesis during heat shock or serum depletion (9). Both phosphorylated and nonphosphorylated forms of eIF4E bind to the mRNA cap structure (33). However, the phosphorylated form possesses a fourfold greater affinity for cap analogs and mRNA than does the unphosphorylated form, providing a potential explanation for the correlation between phosphorylation of eIF4E and rates of protein synthesis (33).

A second mechanism for modulating eIF4E involves its relative distribution between inactive and active complexes with other proteins. eIF4E binds to small, acid- and heat-labile proteins termed 4E-BP1 (PHAS-I), 4E-BP2, and 4E-BP3 to form an inactive complex (32, 39). In rat skeletal muscle, the predominant eIF4E binding protein is 4E-BP1. Hypophosphorylated 4E-BP1 binds to eIF4E to form an inactive 4E-BP1·eIF4E complex. When eIF4E is bound to 4E-BP1, eIF4E binds to mRNA but cannot form an active eIF4E·eIF4G complex (19), thereby preventing binding of mRNA to

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the ribosome. 4E-BP1 binding to eIF4E essentially limits cap-dependent mRNA translation by sequestering eIF4E into an inactive complex. The binding of 4E-BP1 to eIF4E is controlled by phosphorylation of 4E-BP1. Phosphorylation of 4E-BP1 releases eIF4E from the 4E-BP1·eIF4E complex and allows the eIF4E·mRNA complex to bind to eIF4G and, through eIF4G, to the 40S ribosome (39).

At present, there is no information regarding the effect of IGF-I on the regulation eIF4E in skeletal muscle. Therefore, the present set of investigations was designed to examine the mechanisms by which IGF-I regulates translation initiation in skeletal muscle. Specifically, the effect of IGF-I on initiation factors controlling mRNA binding to the ribosome was investigated with a perfused rat hindlimb preparation. The use of the perfused hindlimb preparation allows a direct examination of the effect of IGF-I on the regulation of translation initiation in skeletal muscle independent of alterations in hormones and/or metabolites that occur in vivo after infusion of IGF-I. Furthermore, the perfused hindlimb eliminates the potential effects of the IGF-I binding proteins in modulating IGF-I action. The results indicate that IGF-I increases binding of eIF4E to eIF4G, whereas it has little effect on 4E-BP1 associated with eIF4E.

MATERIALS AND METHODS

Experimental protocols. Adult male Sprague-Dawley rats weighing 150–225 g were maintained on a 12:12-h light-dark cycle and were fed ad libitum until time of perfusion. Experiments were performed with the isolated perfused hindlimb preparation. Hindlimb perfusions were carried out according to the method described by Bylund-Fellenius et al. (5) as modified by Jurasinski and colleagues (23–25). All perfusions were initiated between 10:00 AM and 12:00 PM. Rats were anesthetized with pentobarbital sodium (50 mg/kg body wt), and the skin covering the right and left hindlimbs was removed. The abdominal aorta was cannulated, and perfusion was started at a rate of 0.32 ml··min^{-1}·g^{-1} to the hindlimb musculature (5). The inferior vena cava was then cannulated. The first 50 ml of perfusate passing through the hindlimb were discarded, after which recirculation of the perfusate was begun. After perfusion for an additional 5 min, L-[^{3}H]phenylalanine was introduced into the perfusate to 2 μCi/ml and perfusion was continued for 60 min. After perfusion, gastrocnemius muscles were frozen between aluminum blocks precooled to the temperature of liquid nitrogen or were used directly for analysis of eIFs. A perfusate sample was withdrawn and centrifuged to remove red blood cells. The plasma samples were stored at −20° until analyzed for phenylalanine specific radioactivity as described previously (23–25).

The perfusate (250 ml/hindlimb) consisted of a modified Krebs-Henseleit bicarbonate buffer containing 30% (vol/vol) washed bovine erythrocytes, 4.5% (wt/vol) bovine serum albumin (fraction V), 11 mM glucose, 1 mM phenylalanine, and all other amino acids at normal rat plasma concentrations as previously described (23–25). The medium was maintained at 37°C and gassed with humidified 95% O_2-5% CO_2. IGF-I was added to give a final concentration of 10 nM. Previous studies have shown that protein synthesis is maximally stimulated at this concentration of the hormone in both perfused hindlimb (25) and incubated epitrochlearis (49). Furthermore, Dardevet et al. (6) have shown that at a concentration of 10 nM, IGF-I neither activates nor cross-reacts with the insulin receptor in muscles composed of fast-twitch fibers.

Measurement of synthesis of total mixed proteins. Rates of protein synthesis were estimated by the incorporation of L-[^{3}H]phenylalanine into muscle proteins (23–25). The rate of protein synthesis, expressed as nanomoles of phenylalanine incorporated into protein per hour per gram of muscle, was determined by dividing the disintegrations per hour incorporated by the perfusate phenylalanine specific radioactivity (23–25). The assumption in using this technique to estimate the rate of protein synthesis is that the intracellular phenylalanine concentration is elevated to high concentrations, thereby limiting any dilution effect of nonradioactive phenylalanine derived from proteolysis. Bylund-Fellenius et al. (5) and Jefferson et al. (21) have provided evidence that at perfusate concentrations above 800 nmol/ml, the specific radioactivity of tRNA-bound phenylalanine is the same as that of the extracellular and intracellular pools of free phenylalanine. Therefore, the specific radioactivity of the perfusate phenylalanine provides an accurate estimate of the specific radioactivity of phenylalanyl t-RNA.

Total RNA content and measurement of translational efficiency. Total RNA was measured in homogenates from muscle samples as previously described (50, 51). Total RNA was expressed as milligrams of RNA per gram of tissue. Translational efficiency was calculated by dividing the rate of protein synthesis by the total tissue RNA content (5, 50, 51).

Quantification of 4E-BP1·eIF4E and eIF4G·eIF4E complexes. The association of eIF4E with 4E-BP1 and eIF4G was determined as previously described (29, 46, 53). eIF4E and the 4E-BP1·eIF4E and eIF4G·eIF4E complexes were quantitatively immunoprecipitated from muscle extracts with a monoclonal antibody to eIF4E. Proteins in the immunoprecipitates were resolved by SDS-polyacrylamide gel electrophoresis, and the proteins were then electrophoretically transferred to a polyvinylidene difluoride membrane as previously described (29, 46, 53). The membranes were incubated with a mouse anti-human eIF4E antibody, a rabbit anti-rat 4E-BP1 antibody, or a rabbit anti-eIF4G antibody for 1 h at room temperature. The blots were then developed with an enhanced chemiluminescence Western blotting kit as per the instructions of the manufacturer (Amersham). Films were scanned with a Microtek ScanMaker III. Images obtained with the ScanWizard Plugin (Microtek) for Adobe Photoshop were quantitated with National Institutes of Health Image 1.60 software.

Determination of phosphorylation state of 4E-BP1. The various phosphorylated forms of 4E-BP1 were measured after immunoprecipitation of 4E-BP1 from muscle homogenates after centrifugation at 10,000 *g* (29, 46). The immunoprecipitates were solubilized with SDS sample buffer. The various phosphorylated forms of 4E-BP1 were separated by electrophoresis and quantitated by protein immunoblot analysis as described previously (26, 28, 29, 53).

Determination of the phosphorylation state of eIF4E. Phosphorylated and unphosphorylated forms of eIF4E in homogenates of gastrocnemius were separated by isoelectric focusing on a slab gel and quantitated by protein immunoblot analysis, as described previously (29, 46, 53).

Statistical analysis. The experimental data for each condition are summarized as means ± SE. The statistical evaluation of the data was performed with Student's *t*-test to test for overall differences between means, which were considered significant when $P < 0.05$.

RESULTS

Rates of protein synthesis in gastrocnemius were stimulated over 2.7-fold in the presence of IGF-I (Fig. 1, *top*). Inclusion of IGF-I in the perfusate did not alter total RNA content (Fig. 1, *middle*) but enhanced translational efficiency ~2.8-fold (Fig. 1, *bottom*).

In previous reports, we suggested that one mechanism responsible for the stimulation of translational efficiency induced by insulin was an increased phosphorylation of 4E-BP1 (28, 29). Phosphorylation of 4E-BP1 is thought to regulate translation initiation by causing dissociation of the 4E-BP1·eIF4E complex (12) with a concomitant decrease in the amount of 4E-BP1 bound to eIF4E. Therefore, we investigated whether or not a similar mechanism could account for stimulation of protein synthesis by IGF-I in perfused skeletal muscle (Fig. 2). 4E-BP1 possesses multiple phosphorylation sites, phosphorylation of which results in reduced mobility after electrophoresis. 4E-BP1 can be resolved into multiple electrophoretic α-, β-, and γ-bands, representing differentially phosphorylated forms of the protein (32, 39). The most highly phosphorylated form of the protein, the γ-form, does not bind eIF4E (32, 39). In the present study, inclusion of IGF-I in the perfusate did not affect phosphorylation of 4E-BP1 (Fig. 2).

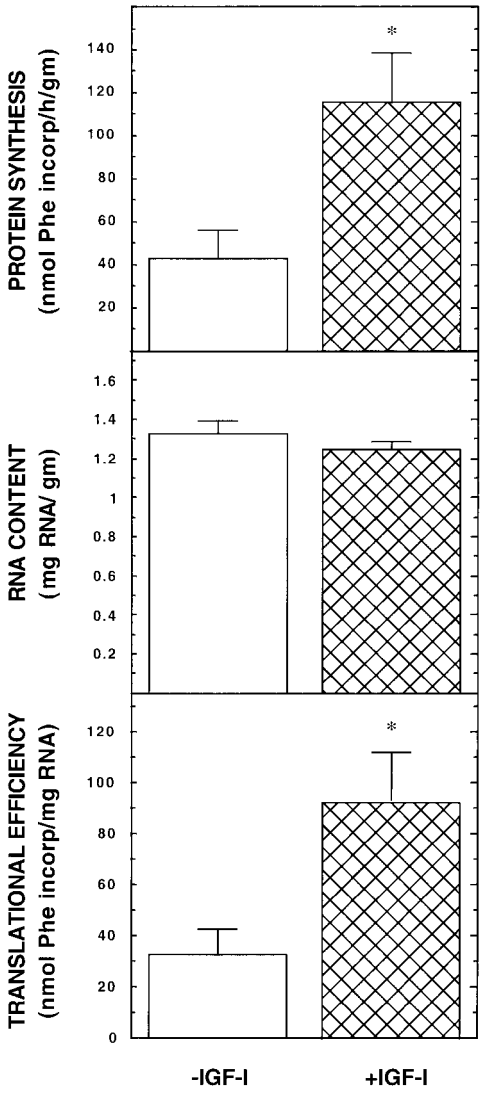

Fig. 1. Effect of insulin-like growth factor I (IGF-I) on protein synthesis (*top*), total RNA content (*middle*), and translational efficiency (*bottom*) in gastrocnemius. Rat hindlimbs were perfused as described in MATERIALS AND METHODS in presence and absence of IGF-I (10 nM). Rates of protein synthesis were estimated by measuring incorporation (incorp) of [^{3}H]phenylalanine into mixed proteins of gastrocnemius as described in MATERIALS AND METHODS. Total RNA was measured in extracts after alkaline hydrolysis as described in MATERIALS AND METHODS. Translational efficiency was calculated by dividing rate of protein synthesis by the RNA content. Results are means ± SE for 6 animals in each group (*$P < 0.05$ vs. IGF-I).

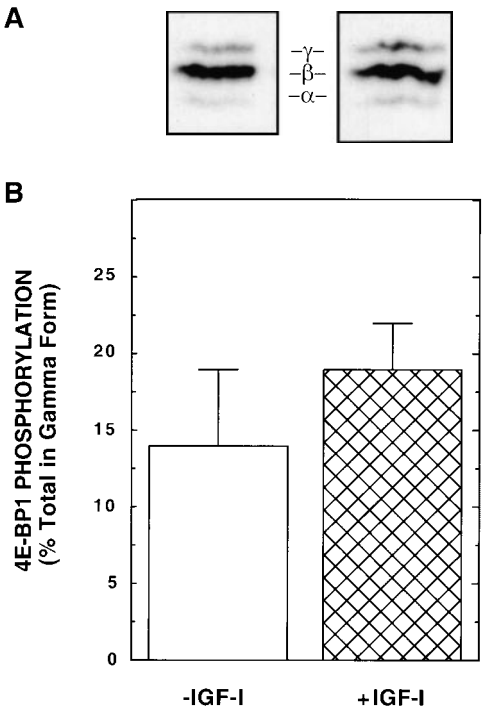

Fig. 2. Effect of IGF-I on phosphorylation of the eukaryotic initiation factor (eIF) 4E binding protein 4E-BP1. Phosphorylation of 4E-BP1 was determined by protein immunoblot analysis after SDS-PAGE from extracts of gastrocnemius obtained from perfusions described in Fig. 1. Amount of 4E-BP1 in γ-phosphorylated form is expressed as a percentage of sum of phosphorylated and nonphosphorylated forms. Results are means ± SE for 5–6 animals in each group. *A*: samples from representative Western blots. Positions of α-, β-, and γ- forms of 4E-BP1 are noted. *B*: immunoblot analysis of all Western blots as described in MATERIALS AND METHODS.

Even though phosphorylation of 4E-BP1 was unaffected, it remained a possibility that IGF-I modulated the association of 4E-BP1 with eIF4E. To investigate the effect of IGF-I on the association of 4E-BP1 with eIF4E, eIF4E immunoprecipitates were analyzed for 4E-BP1 content (Fig. 3). Inclusion of IGF-I in the perfusate had no significant effect on the amount of 4E-BP1 associated with eIF4E.

In a similar manner, eIF4E immunoprecipitates were also used to measure the association of eIF4G with eIF4E (Fig. 4). IGF-I caused a threefold increase in the amount of eIF4G that was immunoprecipitated with eIF4E. This observation was not the result of an

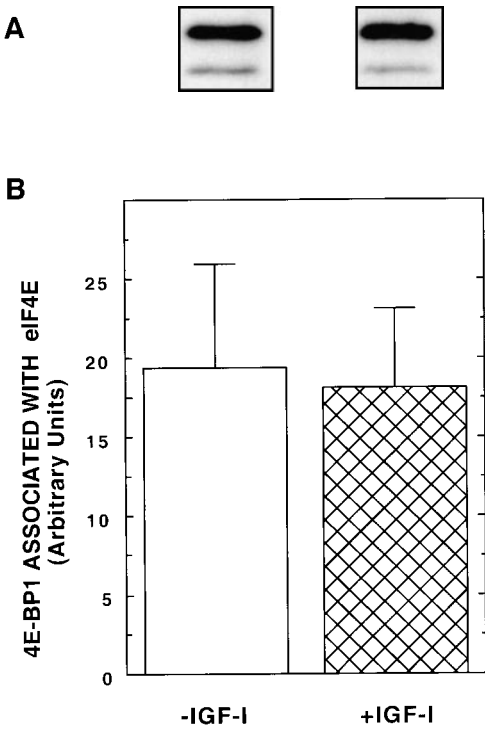

Fig. 3. Effect of IGF-I on amount of 4E-BP1 bound to eIF4E. To determine amount of 4E-BP1 bound to eIF4E, eIF4E was immunoprecipitated from 10,000 *g* extracts of gastrocnemius obtained from perfusions described in Fig. 1 by use of an anti-eIF4E monoclonal antibody. Immunoprecipitates were subsequently subjected to protein immunoblot analysis with anti-4E-BP1 antibodies. Results are means ± SE for 5–6 animals in each group. *A*: samples from representative Western blots. *B*: immunoblot analysis of all Western blots as described in MATERIALS AND METHODS.

increased amount of eIF4E in the immunoprecipitates (data not shown). This finding indicates that IGF-I may stimulate translation efficiency, and hence protein synthesis, in skeletal muscle, in part by promoting formation of the active eIF4E·eIF4G complex.

Finally, we examined the effect of IGF-I on phosphorylation of eIF4E (Fig. 5). There was no significant change in the extent of eIF4E phosphorylation when IGF-I was included in the perfusate.

DISCUSSION

IGF-I accelerates the rate of protein synthesis in skeletal muscle by enhancing translational efficiency. However, the mechanism responsible for the stimulation of translational efficiency by IGF-I is presently unknown. The present investigation is the first to address the role of initiation factors in the stimulation of protein synthesis by IGF-I in skeletal muscle. We investigated the ability of IGF-I to modulate the binding of eIF4E to eIF4G, a crucial step controlling translation initiation in skeletal muscle. We observed that IGF-I enhanced the amount of eIF4E bound to eIF4G in perfused skeletal muscle. During translation initiation, mRNA binds either directly to eIF4E already associated with 40S ribosomal subunits or to free eIF4E with subsequent binding of the mRNA·eIF4E·eIF4G complex to the ribosome. With either scenario, the increased amount of eIF4E associated with eIF4G after perfusion with IGF-I would result in an increased association of mRNA with the ribosome (41, 45). Thus IGF-I may stimulate protein synthesis in part by improving the ability of eIF4E to bind mRNA to the 43S preinitiation complex through an increased abundance of the active eIF4E·eIF4G complex in skeletal muscle.

Both diabetes and starvation increase the amount of eIF4E found in the inactive 4E-BP1·eIF4E complex in skeletal muscle, with a concomitant decrease in the association of eIF4E with eIF4G (28, 29). Conversely, refeeding of starved rats or insulin treatment of diabetic rats causes a dissociation of the 4E-BP1·eIF4E complex, thereby promoting the association of eIF4E with eIF4G (28, 29, 46). In this simplistic model, the release of eIF4E from the 4E-BP1·eIF4E complex allows eIF4E to bind to eIF4G and form the active eIF4G·eIF4E complex. Indeed, stimulation of protein synthesis in perfused skeletal muscle or L6 muscle cells in culture in response to acute administration of insulin is associated with an increase in the amount of eIF4E bound to eIF4G (27, 29). In the present set of experiments, IGF-I did not diminish the abundance of eIF4E associated with 4E-BP1. Thus we did not observe a reciprocal relationship between eIF4E found in the inactive 4E-BP1·eIF4E complex and that in the active eIF4G·eIF4E complex. Hence, protein synthesis

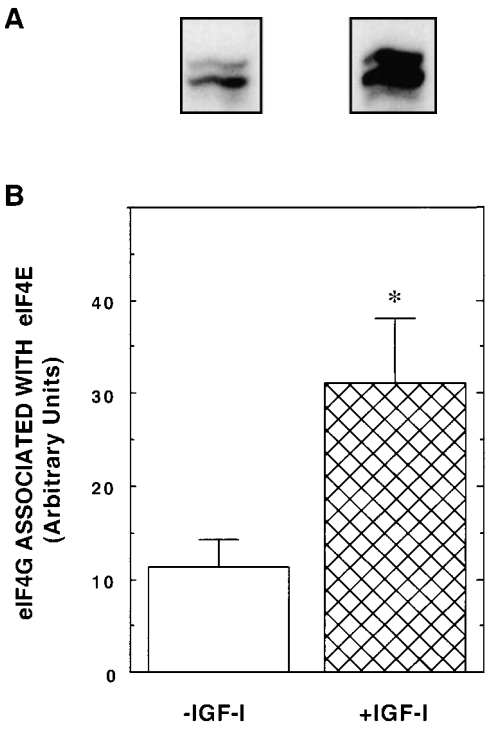

Fig. 4. Effect of IGF-I on amount of eIF4G bound to eIF4E. To determine amount of eIF4G bound to eIF4E, eIF4E was immunoprecipitated from 10,000 *g* extracts of gastrocnemius obtained from perfusions described in Fig. 1 by use of an anti-eIF4E monoclonal antibody. Immunoprecipitates were subsequently subjected to protein immunoblot analysis with anti-eIF4G antibodies. Results are means ± SE for 5–6 animals in each group. *A*: samples from representative Western blots. *B*: immunoblot analysis of all Western blots as described in MATERIALS AND METHODS (* $P < 0.05$ vs. -IGF-I).

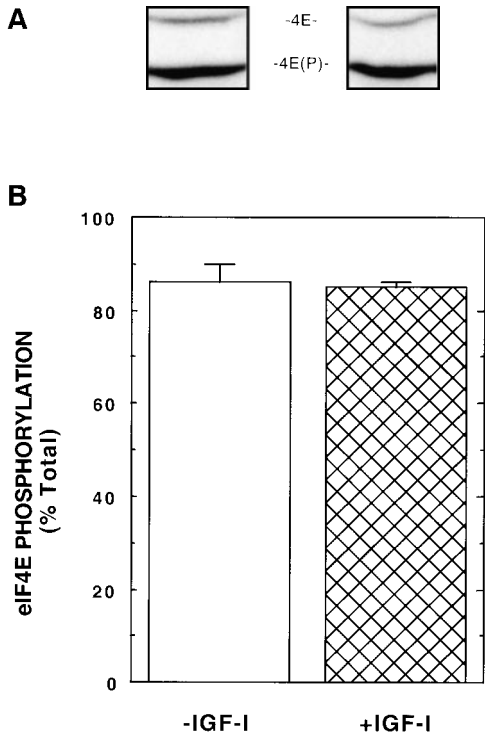

Fig. 5. Effect of IGF-I on the phosphorylation of eIF4E. Phosphorylation of eIF4E was determined by protein immunoblot analysis after isoelectric focusing from extracts of gastrocnemius obtained from perfusions described in Fig. 1. Amount of eIF4E in phosphorylated form is expressed as a percentage of the sum of phosphorylated and nonphosphorylated forms. *A*: samples from representative Western blots. Positions of phosphorylated [4E(P)] and nonphosphorylated (4E) forms of eIF4E are noted. *B*: immunoblot analysis of all Western blots as described in MATERIALS AND METHODS. Results are means ± SE for 5–6 animals in each group.

appears to be augmented by IGF-I without reductions in the abundance of the 4E-BP1·eIF4E complex.

The interaction between 4E-BP1 and eIF4E is regulated by the extent of 4E-BP1 phosphorylation. Phosphorylation of 4E-BP1 releases eIF4E from the 4E-BP1·eIF4E complex (39). In the present study, perfusion with IGF-I did not significantly modulate the phosphorylation of 4E-BP1. Because 4E-BP1 phosphorylation was unaltered, it is not surprising that IGF-I did not affect abundance of eIF4E associated with 4E-BP1.

4E-BP1 is phosphorylated in vitro by a kinase that is referred to as the mammalian target of rapamycin (mTOR) (3, 4). The mTOR pathway does not appear to be the predominant pathway responsible for the stimulation of protein synthesis by IGF-I in skeletal muscle. The specific inhibition of the mTOR pathway with rapamycin results in only a partial reduction (−20%) in the IGF-I-induced stimulation of protein synthesis (7). Thus the rapamycin-insensitive component of IGF-I signaling appears to be the more important pathway responsible for the stimulation of protein synthesis by IGF-I in intact skeletal muscle. Hence, it is not surprising that IGF-I was able to stimulate translation initiation without enhancing the phosphorylation of 4E-BP1.

At least two other reports indicate that the association of eIF4E with eIF4G can be modulated independent of eIF4E binding to 4E-BP1 (14, 34). In both NIH 3T3 cells (34) and *Xenopus* kidney cells (14) in culture, serum promoted protein synthesis and increased association of eIF4E with eIF4G without any alteration in the amount of eIF4E bound to 4E-BP1. Moreover, several studies have shown a poor temporal correlation between inhibition of phosphorylation of 4E-BP1, the inhibition of general translation initiation, and eIF4E binding to eIF4G (3, 14, 26, 44). Thus physiological concentrations (10 nM) of IGF-I in skeletal muscle also appear to modulate the binding of eIF4E to eIF4G independent of changes in the association of eIF4E with 4E-BP1.

Our results are in contrast to previous reports concerning the effect of IGF-I on 4E-BP1 in aortic smooth muscle cells (17) or RINm5F cells (52) in culture. In those investigations, IGF-I increased the phosphorylation state of 4E-BP1 and promoted a dissociation of the inactive 4E-BP1·eIF4E complex. There are numerous reasons for the apparent discrepancies between results obtained with cells in culture and our results in perfused skeletal muscle. First, there may be inherent distinctions in the sensitivity of skeletal muscle to IGF-I compared with other organs. Second, the differences may result from disparity of response associated with cells in culture vs. intact skeletal muscle. For example, both the aortic smooth muscle cells (17) and RINm5F cells (52) were incubated in DMEM. The concentrations of individual amino acids in DMEM (13) are 1.5- to 15-fold higher than those used in the perfusate. The higher concentration of amino acids, particularly leucine, may sensitize the signaling pathway for IGF-I-induced phosphorylation of 4E-BP1. In this regard, elevating amino acid concentrations, and leucine in particular, enhances the ability of IGF-I to phosphorylate 4E-BP1 in RINm5F cells in culture (52). Third, the lack of effect of IGF-I on the phosphorylation of 4E-BP1 may result from the less than maximal stimulation of upstream regulators of mTOR activity by IGF-I at the perfusate concentration (10 nM) used. The binding of IGF-I to its receptor induces autophosphorylation of the receptor and promotes phosphorylation of insulin receptor substrate-1. Insulin receptor substrate-1 serves as a docking protein for proteins containing SH2 domains such as the p85 subunit of phosphatidylinostitol (PI) 3-kinase. PI 3-kinase acting through a cascade of kinases can activate mTOR. Signaling downstream of mTOR includes the p70 S6 kinase and 4E-BP1 phosphorylation. The failure of IGF-I to fully activate mTOR pathway is not the result of a generalized unresponsiveness of this pathway during in vitro perfusion, because insulin enhanced the phosphorylation of both p70 S6 kinase (T. C. Vary and S. R. Kimball, unpublished data) and 4E-BP1 (29) under similar perfusion conditions. Instead, the low concentration of IGF-I used in the present set of experiments may be insufficient to activate this pathway. Approximately 10-fold higher IGF-I concentrations are required to obtain similar effects on PI 3-kinase activity as insulin in skeletal muscle (22). Thus IGF-I concentrations as high as 500 nM are

required for maximal stimulation of PI 3-kinase observed at physiological insulin concentrations (50 μU/ml) (22). At the concentrations used in the present set of experiments (10 nM), it is possible that increases in PI 3-kinase activity are insufficient to induce activation of the mTOR signaling pathway. Lastly, it is possible that IGF-I preferentially modulates the phosphorylation of 4E-BP2 or 4E-BP3 and not of 4E-BP1.

Phosphorylation of eIF4E promotes the binding of the initiation factor to the m^7GTP cap structure and correlates with acceleration of protein synthesis in a variety of cells in culture stimulated with growth factors, mitogens, and anabolic hormones (40, 45). However, we were unable to detect a significant increase in phosphorylation of eIF4E at a time when protein synthesis was stimulated by IGF-I. Likewise, insulin failed to increase the phosphorylation of eIF4E in perfused hindlimb at a time when protein synthesis was enhanced (29).

In conclusion, IGF-I may stimulate protein synthesis in skeletal muscle primarily by enhancing the binding of eIF4E to eIF4G. The change in the binding of eIF4E to eIF4G (threefold) is similar in magnitude to the stimulation of protein synthesis (2.7-fold). The increased association of eIF4G with eIF4E occurred independent of a reduction in the inactive 4E-BP1·eIF4E complex. Furthermore, the findings indicate that formation of the active eIF4G·eIF4E complex is more complicated than simply a release of eIF4E from the inactive 4E-BP1·eIF4E complex and its subsequent association with eIF4G.

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