Tissue-specific regulation of protein synthesis by insulin and free fatty acids


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STARVATION and experimentally induced diabetes, both conditions of insulin deficiency, are associated with an inhibition of protein synthesis in rat skeletal muscle and heart (34, 38). The inhibition is more severe in skeletal muscles consisting of a high proportion of fast-twitch, glycolytic fibers than in heart or skeletal muscles consisting predominantly of slow-twitch, oxidative fibers (17, 34). The difference in severity between the two types of tissues has been ascribed to a combination of an inhibition in mRNA translation and a reduction in ribosomal RNA in fast-twitch skeletal muscle in contrast to heart or slow-twitch skeletal muscle, in which the inhibition is due largely to a loss of ribosomal RNA (33). These findings suggest that translational control of protein synthesis is differentially regulated in glycolytic compared with oxidative skeletal and cardiac muscle fibers.

In perfused hindlimb preparations from starved or diabetic rats, addition of insulin to the perfusate reverses the inhibition of translation initiation and largely restores protein synthesis in predominantly fast-twitch skeletal muscle such as the gastrocnemius to the rate observed in control animals (9). In contrast, addition of free fatty acids (FFAs) to perfused hindlimb preparations from starved or diabetic animals has no effect on translation initiation in fast-twitch skeletal muscle (17). In perfused heart preparations, deprivation of insulin causes a defect in translation initiation similar to that seen in fast-twitch skeletal muscle of starved or diabetic animals. However, the defect in heart can be reversed by addition of either insulin or FFAs to the perfusate (37). These findings have led to the suggestion that plasma FFAs are involved in maintaining translation initiation in the heart (17, 36). This suggestion is supported by the recent observation that administration of an inhibitor of FFA oxidation results in an inhibition of translation initiation within the heart (6). In that study, suppression of β-oxidation caused significant changes in the function of proteins involved with the initiation of mRNA translation, i.e., eukaryotic initiation factors (eIFs), in the heart but not in the gastrocnemius. Furthermore, ATP-to-AMP ratios were significantly reduced in the heart, but not the gastrocnemius, after the suppression of β-oxidation, suggesting that cellular energy status may influence translation initiation rates in the heart.

Translation initiation is a highly orchestrated process wherein an initiator methionyl tRNA (tRNA^Met) and an mRNA join with the two ribosomal subunits to form a translationally competent 80S ribosomal initiation complex (27). The process is responsive to environmental, nutritional, and/or hormonal cues and can be regulated at several steps. Two regulatory steps in particular, those involving the mammalian target of
rapamycin (mTOR) and eukaryotic initiation factor 2B (eIF2B), have key roles in modulating the initiation process in skeletal muscle. The protein kinase mTOR is believed to mediate insulin-dependent effects on translation initiation (30), at least in part, by regulating the phosphorylation, and thus the function, of the eIF4E-binding protein (4E-BP1) and the ribosomal protein S6 kinase (S6K1) (3, 4). The guanine nucleotide exchange factor eIF2B catalyzes the exchange of eIF2-bound GDP for GTP, thus facilitating the transfer of tRNA^Met to the 40S ribosomal subunit by eIF2. The activity of eIF2B correlates with protein synthetic rates under both physiological and pathophysiological conditions (18), and, like mTOR, eIF2B activity in glycolytic skeletal muscle appears to be modulated by circulating insulin concentrations (16, 21).

Presently there is little information regarding the regulation and thus the roles of mTOR signaling and eIF2B activity in modulating translation initiation in the heart. Therefore, one objective of the study described herein was to investigate the roles of these two regulatory processes in the maintenance of protein synthesis in the heart during periods of altered FFA and insulin availability. We employed a rat model of experimental diabetes, wherein circulating insulin concentrations are decreased but FFA concentrations are increased, and a niacin-treated rat model, wherein both circulating FFA and insulin concentrations are simultaneously decreased (41), to examine the contributions of insulin and FFAs to the regulation of mTOR signaling and eIF2B activity in vivo. An additional objective of the study was to discern whether or not tissue-specific differences in mTOR signaling and eIF2B activity correlate with maintenance of cellular energy status during periods of altered FFA and insulin availability.

RESEARCH DESIGN AND METHODS

Animal care. The animal facilities and protocol were reviewed and approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine. The protocol involved the use of male Sprague-Dawley rats (200 g) maintained on a 12:12-h light-dark cycle with a standard diet (Harlan-Teklad Rodent Chow, Madison, WI) and water provided ad libitum.

Experimental diabetes. Three days before experimentation, 16-h food-deprived rats randomly selected for induction of diabetes were administered an intravenous injection of alloxan (48 mg/kg body wt in 0.9% NaCl; Sigma, St. Louis, MO). The remaining animals received an equivalent volume of sterile saline via intravenous injection. Animals were re-allowed free access to water only. Fifty minutes after the third niacin or saline injection, animals were administered a metabolic tracer.

Administration of metabolic tracer and sample collection. A flooding dose (1.0 ml/100 g body wt) of [-2,3,4,5,6-3H]phenylalanine (150 nmol/l, containing 3.70 GBq/l) was administered via tail vein injection 50 min after the final intraperitoneal injection of niacin or saline for the measurement of protein synthesis in skeletal and cardiac muscle (10). Animals were killed 10 min later by decapitation using a rodent guillotine (Kent Scientific, Torrington, CT). Trunk blood was collected and centrifuged at 1,800 g for 10 min at 4°C to obtain serum. The hindlimb muscle, consisting of red and white gastrocnemius and plantaris muscles (hereafter referred to as gastrocnemius), and the heart were excised. The gastrocnemius and a portion of the heart were then homogenized in 7 volumes of buffer consisting of (in mM) 20 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (pH 7.4), 100 KCl, 0.2 EDTA, 2 EGTA, 1 dithiothreitol, 50 sodium fluoride, 50 β-glycerophosphate, 0.1 phenylmethylsulfonyl fluoride, 1 benzamidine, and 0.5 sodium vanadate. An aliquot (0.5 ml) of the homogenate was used for the measurement of protein synthesis as described below. The remainder of the homogenate was immediately centrifuged at 10,000 g for 10 min at 4°C. The supernatant was used for assessment of mRNA translation initiation factor, as described in Assessment of mTOR-mediated signaling and Phosphorylation of eIF2α, and protein kinase phosphorylation state as described in Phosphorylation of AMP-activated protein kinase. The remaining tissue was divided and either snap-frozen in liquid nitrogen for measurement of RNA content, as described in Quantification of tissue RNA content, or used to quantify eIF2B activity as described in Measurement of eIF2B activity.

Serum measurements. Serum insulin concentrations were analyzed using a commercial radioimmunoassay kit for rat insulin (Linco Research, St. Charles, MO), and serum FFAs were analyzed using a commercial colorimetric FFA kit (Wako Chemicals, Richmond, VA). Serum glucose concentrations were measured using an automated glucose oxidase-peroxidase method (YSI model 2300 analyzer, Yellow Springs, OH).

Quantification of tissue RNA content. RNA was extracted from muscle and quantified as described previously (11, 29).

Measurement of muscle protein synthesis. Fractional rates of muscle protein synthesis (nmol Phe*mg protein⁻¹·h⁻¹) were estimated from the rate of incorporation of radioactive phenylalanine into protein as described previously (23). The elapsed time from injection of the metabolic tracer until homogenization of muscle was recorded as the actual time for radiolabeled phenylalanine incorporation. Protein synthetic efficiency (nmol Phe·mg RNA⁻¹·h⁻¹) was determined by dividing the rates of protein synthesis by the amount of RNA per milligram of tissue protein (mg RNA/mg protein) as described previously (9).

Assessment of mTOR-mediated signaling. Phosphorylation of mTOR was evaluated in 10,000-g supernatants by protein...
immunoblot analysis. Proteins in each sample were resolved by SDS-PAGE on a 6% (wt/vol) polyacrylamide gel. A standard Western blot was then performed, and the blots were incubated with an antibody against the phosphorylated form of mTOR (Ser2448, Cell Signaling, Beverly, MA). Proteins were visualized by enhanced chemiluminescence (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). Blots were stripped and reanalyzed using an antibody that recognizes both the phosphorylated and unphosphorylated forms of the protein (Cell Signaling). Phosphorylation of two downstream targets of mTOR, 4E-BP1 and S6K1, was determined in 10,000-g supernatants by protein immunoblot analysis, as described previously (12, 22).

Measurement of eIF2B activity. The guanine nucleotide exchange activity of eIF2B in cardiac muscle was assessed by the exchange of [3H]GDP bound to eIF2 for nonradioactively labeled GDP, as described previously (21).

Phosphorylation of eIF2α. Phosphorylation of eIF2α was determined in 10,000-g supernatants by protein immunoblot analysis, as described previously (23).

Phosphorylation of AMP-activated protein kinase. Phosphorylation of the catalytic subunit of AMP-activated protein kinase (AMPK) was evaluated in 10,000-g supernatants by protein immunoblot analysis. Proteins in each sample were resolved by SDS-PAGE on a 10% (wt/vol) polyacrylamide gel. A standard Western blot was then performed, and the blots were incubated with an antibody against the phosphorylated form of AMPK (Thr172, Cell Signaling). Proteins were visualized by enhanced chemiluminescence (Amersham Biosciences). Blots were stripped and reanalyzed using an antibody that recognizes both the phosphorylated and unphosphorylated forms of the protein (Cell Signaling).

Determination of NADP+-to-NADPH ratios. NADP+-to-NADPH (NADP+/NADPH) ratios were calculated in the heart and gastrocnemius from the ratio of tissue malate to pyruvate, as described previously (18).

Statistical analysis. Data are expressed as means ± SE. All data were analyzed by the InStat version 3 statistical software package (GraphPad Software, San Diego, CA) using separate ANOVA for diabetes and niacin treatment. When ANOVA indicated a significant overall effect, differences among individual means were assessed using Tukey’s post-test. P values of <0.05 were considered statistically significant.

RESULTS

Serum insulin, FFA, and glucose concentrations were quantified to assess the efficacy of alloxan-induced diabetes and niacin administration. As can be seen in Table 1, serum insulin concentrations were decreased to 9 and 29% of control values after induction of diabetes or niacin administration, respectively. The decrease in serum insulin concentrations after niacin administration is in accord with previous studies and demonstrates the essentiality of FFA for insulin secretion in fasted animals (26). The effects of diabetes and niacin on circulating FFA and glucose concentrations (Table 1) were significantly different. Whereas FFA concentrations in diabetic animals were nearly twofold greater than those of controls, rats administered niacin had concentrations that were reduced to <20% of control values. Moreover, glucose concentrations were more than fourfold greater than those of controls in diabetic animals but were not significantly altered in niacin-treated animals.

As reported previously (9, 33), protein synthetic rates in both the heart and the gastrocnemius (data not shown) of diabetic animals were decreased, with the greater change occurring in the gastrocnemius. Furthermore, the decreases in protein synthesis could be attributed, in part, to an ∼15% reduction in RNA content in either tissue (Fig. 1A). When expressed as protein synthesis per RNA or protein synthetic efficiency (Fig. 1B), it is evident that there is a significant decline in both skeletal and cardiac muscle protein synthesis that was due, in large part, to changes in translational control. Furthermore, the inhibition of protein synthetic efficiency was significantly greater in the gastrocnemius than in the heart. These results are in keeping with the observations reported by Flaim et al. (9) and Pain et al. (33) with regard to fast-twitch glycolytic fibers, but not with regard to cardiac muscle fibers, from acutely diabetic animals.

There was no change in RNA content of either the gastrocnemius or heart (Fig. 1A) after niacin administration. Changes in protein synthetic efficiency (Fig. 1B) were therefore due entirely to changes in translational control and, in contrast to diabetic animals, synthesis changes were not significantly different between the heart and the gastrocnemius after niacin administration.

Because alterations in mTOR-mediated signaling and eIF2B activity correlate with protein synthetic rates in skeletal muscle (21, 40), we subsequently investigated how alterations in FFA and insulin availability influence these regulatory mechanisms in skeletal and cardiac muscle. Phosphorylation of mTOR at Ser2448 (Fig. 2), which is associated with increased mTOR protein kinase activity in mitogen-stimulated cells (39), was less than control values in the heart and the gastrocnemius of diabetic animals, suggestive of a decrease in mTOR-mediated signaling in both tissues. Similarly, mTOR phosphorylation was less than control values in the heart and gastrocnemius of niacin-treated animals. However, the effect of niacin was greater in the heart than in the gastrocnemius, indicative of tissue-specific alterations in mTOR-mediated signaling after niacin administration.

To pursue the suggestion of decreased mTOR-mediated signaling in diabetic or niacin-treated animals,
the phosphorylation states of S6K1 and 4E-BP1, two downstream targets of mTOR, were assessed. As with mTOR phosphorylation, the prevalence of hyperphosphorylated S6K1 (Fig. 3A) and 4E-BP1 (Fig. 3B) was decreased in both the heart and the gastrocnemius of diabetic and niacin-treated animals. As with mTOR phosphorylation, the effect of niacin treatment on S6K1 and 4E-BP1 phosphorylation was greater in the heart than in the gastrocnemius. Furthermore, and in contrast to niacin treatment, the effect of diabetes on 4E-BP1 phosphorylation was significantly greater in the gastrocnemius than in the heart. These results are again indicative of FFAs and insulin having tissue-specific alterations in mTOR-mediated signaling.

Consistent with previous reports (21), eIF2B activity (Fig. 4A) was unaltered in hearts of diabetic animals. Previous reports (21) have demonstrated a significant inhibition of eIF2B activity in the gastrocnemius of diabetic animals. A decrease in eIF2B activity was observed in this study but was only significant compared with control values by use of a Student’s t-test. In contrast to diabetic animals, there was a significant inhibition of eIF2B activity in the heart of niacin-treated animals. In addition, eIF2B activity tended to decrease in the gastrocnemius after niacin treatment but, again, was only significant compared with control values by use of a Student’s t-test.

Multiple mechanisms exist for the modulation of eIF2B activity, including the phosphorylation of the α-subunit of eIF2 (eIF2α) and through alterations in the redox state of pyridine dinucleotides. When phosphorylated at Ser51, eIF2α is able to sequester eIF2B into an inactive complex (31), and as such, eIF2α phosphorylation is often associated with decreased protein synthetic rates (20, 32). However, as has been described previously (5, 18), phosphorylation of eIF2α at Ser51 (Fig. 4B) was unaltered in the heart and gastrocnemius of diabetic animals. The effect of niacin treatment on eIF2α phosphorylation was significantly different in the gastrocnemius and the heart. Although phosphorylation was unaltered in the gastrocnemius, there was a significant increase in Ser51 phosphorylation in the heart. In a previous study (18), it was demonstrated that the NADP+/NADPH ratio was unchanged in gastrocnemius of diabetic compared with control rats but was decreased to $<30\%$ of the control
value in the hearts of diabetic rats. Thus it was postu-
lated that the significantly reduced NADP+/NADPH ratio observed in the diabetic rat heart prevents allo-
steric inhibition of eIF2B and thus preserves eIF2B activity (18). In keeping with this postulate, the
NADP+/NADPH ratio (data not shown) was unaltered
in the gastrocnemius of niacin-treated animals but was
2.5-fold greater in the niacin-treated rat heart com-
pared with control hearts. The results above indicate,
as with mTOR-mediated signaling, that the regulation
of eIF2B activity by FFAs and insulin varies in a
tissue-specific manner.

AMPK is believed to respond to changes in cellular
energy status, and its activation is demarcated by
phosphorylation at Thr172 (reviewed in Refs. 13, 14). As
such, Thr172 phosphorylation was assessed in diabetic

Fig. 3. A: hyperphosphorylated ribosomal protein S6 kinase (S6K1) in cardiac and gastrocnemius muscles of control, diabetic, and niacin-treated rats. When subjected to SDS-PAGE, S6K1 is resolved into multiple electrophoretic forms whereby the most highly phospho-
rylated forms exhibit the slowest electrophoretic mobility (42). Inset: results of typical blots. α, S6K1α; β, S6K1β; γ, S6K1γ. Results are expressed as the ratio of hyperphosphorylated S6K1 (β- and γ-forms) to total S6K1. Values represent means ± SE; n = 5–16. B: phosphorylation of eukaryotic initiation factor (eIF4E-binding protein-1 (eIF4E-BP1) in cardiac and gastrocnemius muscles of control, diabetic, and niacin-treated rats. When subjected to SDS-PAGE,
eIF4E-BP1 is resolved into multiple electrophoretic forms whereby the most highly phosphorylated forms exhibit the slowest electrophoretic mobility (28). Inset: results of typical blots. α, 4E-BP1α; β, 4E-BP1β; γ, 4E-BP1γ. Results are expressed as the inverse ratio of hypophosphorylated 4E-BP1 (α-form) to total 4E-BP1. Values represent means ± SE; n = 4–12. B: phosphorylation of eIF2α on Ser51 in cardiac and gastrocnemius muscles of control, diabetic, and niacin-treated rats. Phosphorylation of the α-subunit of eIF2 was assessed by Western blot analysis using an anti-phospho-eIF2α that specifi-
cally recognizes the protein when it is phosphorylated on Ser51. Total eIF2α content was measured by Western blot analysis using a monoclonal antibody that recognizes phosphorylated and unphos-
phorylated forms of the protein. Inset: results of typical blots. eIF2α(P), eIF2α phosphorylated on Ser51; eIF2α, total eIF2α content. Values represent means ± SE; n = 13–16. Solid bars, heart values; hatched bars, gastrocnemius values; open bars, control animals; light grey bars, diabetic animals; dark grey bars, niacin-treated animals. *Signifi-
cantly different from control value; †tissue-specific effect, P < 0.05.
and unphosphorylated forms of the protein. Total AMPK content was measured by Western blot analysis using a polyclonal antibody that recognizes phosphorylated and unphosphorylated forms of the protein. Inset: results of typical blots. AMPK(P), AMPK phosphorylated on Thr<sup>172</sup>; AMPK, total AMPK content. Values represent means ± SE; n = 8–16. Solid bars, heart values; hatched bars, gastrocnemius values; open bars, control animals; light grey bars, diabetic animals; dark grey bars, niacin-treated animals. *significantly different from control value; †tissue-specific effect, P < 0.05.

and niacin-treated animals. As shown in Fig. 5, diabetesc had a significantly different effect on AMPK phosphorylation in the gastrocnemius and heart. Whereas there was an increase in AMPK phosphorylation in the gastrocnemius of diabetic animals, there was no significant change in the heart. In contrast to diabetes, Thr<sup>172</sup> phosphorylation was significantly increased in the heart, but was unaltered in the gastrocnemius, after niacin administration.

**DISCUSSION**

Conditions of insulin deficiency are characterized by a decline in skeletal muscle protein synthetic rates. This decline results both from a reduction in translation initiation and from a decrease in ribosomal RNA content (9, 33). Decreases in skeletal muscle eIF2B activity and mTOR-mediated signaling have been documented under conditions of insulin deficiency (1, 21) and are believed to be causative for the reductions in both translation initiation and ribosome production. Protein synthetic rates are diminished in the heart during periods of insulin deficiency but not to the same extent as in skeletal muscle (17, 34). As such, differences in the regulation of mTOR-mediated signaling and eIF2B activity between skeletal and cardiac muscle may account for the disparity between protein synthesis inhibition in these tissues during periods of insulin deficiency.

In contrast to previous reports (33, 37), our results do not indicate that the inhibition of mRNA translation initiation that occurs in glycolytic skeletal muscles during periods of insulin deficiency is completely prevented in the heart by the presence of FFAs. Our results do, however, suggest that, in the heart, the presence of FFAs is essential to minimize the inhibition of mRNA translation initiation that occurs during periods of insulin deficiency. The aforementioned discrepancy may be due to differences in experimental models. For example, most of the previous studies were done in vitro, and there may be differences in the sensitivity of protein synthetic measurements made in vivo, compared with those made in vitro, to subtle changes in translation initiation. Moreover, in the study performed by Pain et al. (33), in which in vivo cardiac muscle protein synthesis was unaffected by short-term diabetes, the insulin deficit appears to have been less severe than that observed in the present study.

To our knowledge, this is the first study to demonstrate that, as in skeletal muscle, mTOR-mediated signaling is altered by diabetes in the heart in vivo. Moreover, the significant differences in mTOR, S6K1, and 4E-BP1 phosphorylation state changes between the hearts of diabetic and niacin-treated animals indicate that mTOR-mediated signaling is modulated by both insulin and FFAs in the heart. This conclusion is supported by the recent observation that S6K1 and 4E-BP1 phosphorylation is decreased in the hearts of animals wherein FFA oxidation is inhibited but serum insulin concentrations are unaltered (6) and by the fact that the combined effects of hypoinsulinemia and hyperlipidemia on S6K1 and 4E-BP1 phosphorylation appear to be greater than those of diabetes or inhibited FFA oxidation alone in the heart. It is also pertinent that, despite differences in the duration of insulin deficiencies (4 h vs. 3 days), the effects of niacin-induced hypoinsulinemia and hyperlipidemia were no greater than those of diabetes-induced hypoinsulinemia and hyperlipidemia on S6K1 and 4E-BP1 phosphorylation in the gastrocnemius. These results confirm our previous findings that FFAs do not modulate mTOR-mediated signaling in glycolytic skeletal muscle (6). That mTOR-mediated signaling is modulated by FFAs in a tissue that is highly dependent on oxidative metabolism, but not in a tissue that relies heavily on glycolytic metabolism, suggests that FFA oxidation generates a signal that affects mTOR signaling. It has been postulated that the signaling of mTOR to 4E-BP1 and S6K1 is sensitive to nutritional and energetic sufficiency (7, 35); therefore, changes in cellular energy supply may contribute to the alterations in mTOR-mediated signaling seen in the heart after niacin administration.

The observation that eIF2B activity is decreased after niacin administration supports previous findings that fat metabolism is necessary to maintain guanine nucleotide exchange activity in the heart (6). Karinch et al. (18) suggested that the decreased NADP<sup>+</sup>/NADPH ratio observed in the hearts, but not the gas-
trocnemius, of diabetic rats results from increased fat metabolism and functions, as NADP+ is an inhibitor of guanine nucleotide exchange activity (8), to protect eIF2B activity. This possibility is supported by our observation that, although NADP+/NADPH ratios are unaltered in the gastrocnemius after niacin administration, they are significantly increased in the heart. The observation above, in addition to the finding that eIF2α phosphorylation is increased specifically within the heart after niacin administration, demonstrates that the regulation of eIF2B activity by FFAs is highly tissue specific. Furthermore, the observation from this and previous studies (16, 21) regarding eIF2B activity being decreased in glycolytic muscles, but not the heart, of diabetic rats demonstrates that the regulation of eIF2B activity by insulin is also tissue specific. Given the hypothesis that insulin regulates eIF2B activity in glycolytic muscle, the observation that niacin administration tended to cause a decrease in eIF2B activity in the gastrocnemius was not unexpected. The fact that niacin treatment did not have a greater impact on eIF2B activity in the gastrocnemius may be attributed to the relatively short duration of insulin deficiencies (4 h vs. 3 days in diabetic animals) and to the fact that the severity of insulin deficiency, as assessed by serum insulin concentrations (Table 1), is minor compared with that observed in diabetic animals (significant by Student’s t-test). As such, niacin-induced insulinopenia may not have been adequate to influence eIF2B activity in the gastrocnemius.

AMPK is believed to respond to decreases in cellular energy status (13), and recent publications infer that it modulates protein synthetic rates in both skeletal and cardiac muscle by altering mTOR-mediated signaling (2, 6). In addition, a recent report by Kimura et al. (24) suggested that mTOR and its downstream targets are controlled by AMPK in fibroblast cells. The changes in AMPK phosphorylation observed in the present study suggest that cellular energy stress develops within the gastrocnemius during periods of severe insulin deficiency. We attribute the observation that AMPK activation in the gastrocnemius was significantly altered in diabetic but not niacin-treated animals to the fact that, as described above, the duration of insulin deficiencies was longer and more severe in diabetic animals compared with niacin-treated animals. The hypoinsulinemia experienced by niacin-treated animals may in fact not have been adequate to alter insulin-mediated glucose uptake significantly and as such would not have influenced cellular energy status significantly in the gastrocnemius. This hypothesis is supported by the observation that plasma glucose concentrations (Table 1) were not significantly elevated after niacin treatment. Furthermore, in resting glycolytic skeletal muscle, unlike working cardiac muscle, it is possible that alternate fuels such as ketone bodies would be adequate in maintaining cellular energy status for this relatively short period of time. In contrast to the gastrocnemius, diabetes-induced insulin deficiency is not sufficient for the development of cellular energy stress in the heart. Previously published results indicate that altered FFA metabolism culminates in the activation of AMPK in the heart but not the gastrocnemius (6). Therefore, the change in AMPK phosphorylation observed in the hearts of niacin-treated animals is believed to result from hypolipidemia and not from hypoinsulinemia. In this regard, it is pertinent that the concentration of circulating FFAs (0.26 ± 0.03 mM, n = 7) and AMPK phosphorylation in the heart (127 ± 41% of control value, n = 7) of diabetic animals treated with niacin are intermediate between those values (Table 1 and Fig. 5, respectively) observed in diabetic and niacin-treated nondiabetic animals. The alterations in AMPK phosphorylation observed in this study thus demonstrate that hypoinsulinemia and hypolipidemia culminate in energy stress in the gastrocnemius and heart, respectively. Furthermore, these results suggest that energy stress-induced activation of AMPK contributes to alterations in the translational control of protein synthesis in the gastrocnemius and heart. This hypothesis is supported by our observation that both AMPK phosphorylation and protein synthetic efficiency (61 ± 5% of control value, n = 7) in the hearts of diabetic animals treated with niacin are also intermediate between values (Figs. 5 and 1B, respectively) observed in diabetic and niacin-treated nondiabetic animals. The tight correlation between the degree of AMPK phosphorylation and protein synthetic rates in the heart is perhaps indicative of the sensitivity of the protein synthetic apparatus to changes in cellular energy status in vivo. Although the results of the present study cannot disprove the possibility that AMPK activation affects both mTOR-mediated signaling and eIF2B activity, it is evident that AMPK activation does not regulate eIF2B activity in the gastrocnemius through enhanced eIF2α phosphorylation. Moreover, given that eIF2α phosphorylation was associated with altered NADP+/NADPH ratios, we are intrigued by the possibility of changes in redox potential, or the development of a “redox stress,” may stimulate eIF2α phosphorylation. This idea is not without precedent. Changes in the redox state of the cell are known to influence protein folding, and the accumulation of unfolded protein in the endoplasmic reticulum has been shown to activate the eIF2α protein kinase known as the endoplasmic reticulum resident kinase PERK (15, 19).

These results support the notion that the translational control of protein synthesis is regulated by insulin and FFAs in a tissue-specific manner. There are, however, limitations to our interpretations. First is the fact that the effects of diabetes cannot be attributed solely to changes in FFA and insulin. Additional factors likely contribute to the observed changes in mTOR signaling and eIF2B activity. For instance, glucocorticoids, which are elevated with diabetes, are well-known inhibitors of translation initiation. On the same note, the pharmacological dose of niacin utilized in these studies is likely to have additional systemic effects that have not been addressed here. In view of the results attained, however, the tissue-specific roles of FFAs and insulin in mediating mRNA translation can-
not be overlooked. Second, it should be noted that, although heavily dependent on glycolytic metabolism, the combination of gastrocnemius and plantaris muscle utilized in these experiments consists of multiple fiber types. Additional experiments are therefore required to confirm our assessment of the roles of insulin and FFAs in specific muscle fiber types.

In summary, the results of the present study support the idea that there is a decline in mRNA translation initiation rates in the heart during insulin deficiency. Because of the ability of FFAs to activate both mTOR-mediated signaling and eIF2B activity in the heart, the decline in mRNA translation initiation is not as great as that seen in the gastrocnemius under similar conditions. Furthermore, the results suggest that FFAs are essential for the maintenance of protein synthesis in the heart during insulin deficiency. Similar to the gastrocnemius during insulin deficiency, cellular energy status decreases during FFA deficiency, resulting in AMPK activation and inhibition of the mTOR-signaling pathway. Alterations in FFA metabolism also contribute to the inhibition of the eIF2B-signaling pathway, possibly through redox stress-induced phosphorylation of eIF2α.

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DISCLOSURES

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REFERENCES


