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Severe diabetes prohibits elevations in muscle protein synthesis after acute resistance exercise in rats

MARK J. FEDELE, JAZMIR M. HERNANDEZ, CHARLES H. LANG, THOMAS C. VARY, SCOT R. KIMBALL, LEONARD S. JEFFERSON, AND PETER A. FARRELL
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Fedele, Mark J., Jazmir M. Hernandez, Charles H. Lang, Thomas C. Vary, Scot R. Kimball, Leonard S. Jefferson, and Peter A. Farrell. Severe diabetes prohibits elevations in muscle protein synthesis after acute resistance exercise in rats. *J. Appl. Physiol.* 88: 102–108, 2000.—This study determined whether rates of protein synthesis increase after acute resistance exercise in skeletal muscle from severely diabetic rats. Previous studies consistently show that postexercise rates of protein synthesis are elevated in nondiabetic and moderately diabetic rats. Severely diabetic rats performed acute resistance exercise ($n = 8$) or remained sedentary ($n = 8$). A group of nondiabetic age-matched rats served as controls ($n = 9$). Rates of protein synthesis were measured 16 h after exercise. Plasma glucose concentrations were >500 mg/dl in the diabetic rats. Rates of protein synthesis (nmol phenylalanine incorporated \cdot g muscle $^{-1} \cdot$ h $^{-1}$, means \pm SE) were not different between exercised (117 ± 7) and sedentary (106 ± 9) diabetic rats but were significantly ($P < 0.05$) lower than in sedentary nondiabetic rats (162 ± 9) and in exercised nondiabetic rats (197 ± 7). Circulating insulin concentrations were 442 ± 65 pM in nondiabetic rats and 53 ± 11 and 72 ± 19 pM in sedentary and exercised diabetic rats, respectively. Plasma insulin-like growth factor I concentrations were reduced by 33% in diabetic rats compared with nondiabetic rats, and there was no difference between exercised and sedentary diabetic rats. Muscle insulin-like growth factor I was not affected by resistance exercise in diabetic rats. The results show that there is a critical concentration of insulin below which rates of protein synthesis begin to decline in vivo. In contrast to previous studies using less diabetic rats, severely diabetic rats cannot increase rates of protein synthesis after acute resistance exercise.

hypoinsulinemia; anabolic factors; stress

REGULATION OF PROTEIN synthesis by insulin can be viewed in the context of the amount of insulin available and the stimulus for altered rates of protein synthesis. Severe hypoinsulinemia lasting several days clearly reduces protein synthesis in many tissues, including skeletal muscle (19, 31, 32, 46). This reduction in protein synthesis in skeletal muscle is mechanistically linked to a block in peptide chain initiation (35, 36). In contrast, Garlick et al. (24) demonstrated that low

doses of insulin infused into rats for 1 h caused hypoglycemia but had little effect on rates of protein synthesis in skeletal muscle. Rates of protein synthesis only became elevated at very high infusion rates of insulin. Reducing insulin below basal concentrations did not alter protein synthesis; however, this infusion lasted only 1 h, and Jefferson et al. (32) demonstrated a perfusion-induced block in peptide chain initiation and protein synthesis when an insulin-free perfusate is infused for 2 h. Thus the duration of hypoinsulinemia is an important factor for the regulation of protein synthesis.

Using an in situ bilateral hindlimb perfusion, Fluckey et al. (22) demonstrated that postexercise elevations in rates of protein synthesis were ablated when insulin was omitted from the perfusion medium. That study also verified (4) that elevating insulin concentrations for a short period of time (30 min) in nonstressed animals did not increase protein synthesis. It is possible that a low but critical concentration of insulin must be available for anabolism to occur after exercise. One of our objectives for this study was to determine whether such a critical concentration exists in vivo.

Recent studies demonstrate that rates of protein synthesis are higher after acute moderate-intensity resistance exercise in moderately diabetic rats (17, 18). Whether severely diabetic rats can increase rates of protein synthesis after endurance or resistance exercise has not been investigated. The studies reported here investigated whether severe hypoinsulinemia, in combination with other factors known to alter protein turnover, was associated with an inability to increase protein synthesis after acute moderate-intensity resistance exercise.

METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were used in all experiments; they were housed in temperature- and humidity-controlled holding facilities with lights on at 0700 and off at 1900. Rats were fed ad libitum a standard rodent diet (diet 5001, PMI Feeds), which contained 24% protein, 12% fat, 50% carbohydrate, 7% ash, 6% fiber, and vitamins.

Partial pancreatectomy. On the basis of previous work (15), a partial pancreatectomy procedure was used, and the procedure was modified to include rats that weighed 110–140 g as

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opposed to the weights (90–110 g) suggested by Foglia (23). We find that a larger percentage ($\approx 80\%$) of the animals become diabetic when heavier rats are pancreatectomized. We also used a microcauterizer to eliminate small pancreatic blood vessels and to reduce bleeding during surgery. Sterile conditions were maintained throughout the surgery. Rats were anesthetized using methoxyflurane and were kept on a heated surgical pad. The procedure requires the physical removal of pancreatic tissue from the splenic, duodenal, and pyloric regions while major blood vessels are left intact. This is accomplished using sterile cotton Q-tips. Pancreatic tissue between the bile duct and the duodenum is not removed, since this approximates 10% of the original total pancreatic tissue. In an effort to produce severely diabetic rats, we were fastidious in removing as much pancreatic tissue as possible. At the conclusion of surgery, rats were given ampicillin (5 mg/100 g body wt sc; Sigma Chemical, St. Louis, MO) as an antimicrobial agent. Two weeks after partial pancreatectomy, a tail vein blood sample was obtained in the fed state to determine plasma glucose concentrations with use of a Beckman Glucose Analyzer 2. Rats that were not severely diabetic (<475 mg/dl) were eliminated from the study. Sixteen diabetic rats were randomly assigned to exercise ($n = 8$) or sedentary ($n = 8$) groups. Age-matched nondiabetic sedentary ($n = 15$) and exercised ($n = 6$) control rats were housed and handled in a manner that was identical to that used for diabetic rats, with the exception of surgery and tail vein sampling to verify diabetic status. We previously used sham-operated rats for nondiabetic controls, and there was no difference in rates of protein synthesis or plasma insulin levels between the sham and nonsham controls (17). Therefore, we have subsequently used rats with no surgical manipulation for nondiabetic controls.

Resistance exercise. Details of the exercise protocol have been described previously (20). Briefly, rats were operantly conditioned to touch an illuminated bar low on a Plexiglas exercise cage and then were taught to stand and touch an illuminated bar that was located high on the opposite wall of the cage. Electrical foot shock (<2 mA, 60 Hz) was used to reinforce these movements. Once the learning process was completed (3–4 sessions), weighted vests were strapped over the scapulae, and the rats were required to touch the high bar 50 times during one acute exercise session. We defined acute resistance exercise as four separate sessions with 1 day of rest between sessions. The rats performed 50 repetitions each day with 0.2 (*day 1*), 0.4 (*days 2 and 3*), and 0.6 (*day 4*) g weighted vest/g body wt. Previous work showed that a rat that was naive to the lifting procedure would not lift the 0.6 g/g body wt on the 1st day weights were applied to the vest. This protocol can be considered acute, because it does not result in changes in muscle weight (21). We recognize that some metabolic adaptations may occur as a result of multiple bouts of acute exercise (29). However, the rats in this study were clearly not “trained.” Exercise sessions occurred in the dark (red light) in the late afternoon. Rats that did not perform exercise (sedentary) were placed in the lifting cages at least three times during the week of acute exercise and were given five electric shocks to simulate some of the stress experienced by the exercised groups. One of these shock control sessions occurred 16 h before the determination of rates of protein synthesis.

Rates of protein synthesis. All measurements of rates of protein synthesis occurred 16 h after the last bout of acute resistance exercise. Food was withdrawn from the rats during the last 5 h of this 16-h period. Rats were anesthetized with methoxyflurane, and the left carotid artery and right jugular vein were cannulated. Rats remained unconscious after the

placement of catheters and during the measurement of rates of protein synthesis. Total time between the onset of anesthesia and completion of surgery was 10–15 min. One milliliter of arterial blood was taken to determine plasma concentrations of insulin, insulin-like growth factor (IGF) I, corticosterone, free fatty acids, and glucose. A flooding dose (26) of L-[2,3,4,5,6- 3 H]phenylalanine (1 mCi/rat; Amersham Life Science, Arlington Heights, IL) in unlabeled phenylalanine (150 mM; 1 ml/100 g body wt total volume) was injected immediately after cannulation into the venous catheter over a 15-s period. Arterial blood (1 ml) was taken at 6 and 10 min, and then the gastrocnemius muscle was excised. The superficial white muscle was removed, and the remaining muscle was immediately frozen in liquid nitrogen. Fiber type analysis (data not shown) of this portion of the gastrocnemius suggests that it is most like the “mixed” portion of the gastrocnemius, as described by Armstrong and Phelps (2). Frozen muscles were stored at -70°C until phenylalanine incorporation into TCA-precipitable protein was analyzed using dabsylation of the amino acid and measurement on an HPLC (12). Radioactivity in the phenylalanine peak was measured by liquid scintillation counting with appropriate correction for quench. Protein determinations were made using the biuret method, and rates of muscle protein synthesis were calculated using the method of Garlick et al. (26). Hematocrit and Hb were measured using standard techniques.

Comparison to previous studies. Data were collected in this study for severely diabetic and nondiabetic rats only. In combination with the present data, we used our previously published work on nondiabetic (16–18, 21, 22) and moderately diabetic (17) rats to investigate a possible in vivo relationship between arterial plasma insulin concentrations and rates of protein synthesis. Animal handling, surgery, and experimental timing were identical for all animals used in the data provided in Fig. 3. The data set included exercised and sedentary rats.

Hormone, binding proteins, and free fatty acid assays. Plasma insulin (43) and corticosterone (1) concentrations were determined by RIA. The antibody (no. 1013, Linco Research, St. Charles, MO) used in the rat insulin assay also recognizes other mammalian insulin isoforms but does not cross-react with glucagon, pancreatic polypeptide, somatostatin, or IGF-I. The corticosterone antibody (no. RPA 548, Amersham Life Science) does not cross-react with other steroids ($<3\%$). Corticosterone is first separated from corticosterone-binding globulin by heating, which then allows for its direct measurement (1). Plasma IGF-I was determined by RIA with a modified acid-ethanol (0.25 N HCl-87.5% ethanol) procedure with cryoprecipitation (14, 39). The IGF-I antibody (lot no. AFP4892898, National Hormone and Pituitary Program) does not cross-react with insulin or IGF-II and has a sensitivity of 0.03 ± 0.08 ng/tube, and the intra-assay coefficient of variation is $<5\%$. Gastrocnemius muscles used for IGF-I determinations were extracted using acid homogenization and Sep-Pak C_{18} extraction (14, 39) and then assayed using RIA.

IGF binding protein (IGFBP)-3 in plasma was determined by Western ligand blot analysis, as described by Hossenloop et al. (30) and slightly modified by our laboratory (52). Samples were separated on a 15% (wt/vol) SDS-polyacrylamide gel without reduction of disulfide bonds. The electrophoresed proteins were transferred onto nitrocellulose in Tris-methanol-glycine buffer. Nitrocellulose sheets were washed and then incubated overnight with radiolabeled IGF-I. The nitrocellulose sheets were washed extensively in Tween 20, dried, and autoradiographed with X-ray film (Kodak X-Omat AR, Eastman Kodak, Rochester, NY) and intensifying screens

Table 1. *Physical and physiological characteristics of rats*

Group	<i>n</i>	Weight,* g	Plasma Glucose,* mg/dl	Hematocrit, %	Hb, g/dl	Free Fatty Acids,* meq/l	Corticosterone, ng/ml
Diabetic							
Sedentary	8	298 ± 19	559 ± 11	43 ± 0.8	14.4 ± 0.2	0.49 ± 0.09	465 ± 15
Exercised	8	292 ± 22	549 ± 14	45.5 ± 1.1	14.7 ± 0.3	0.44 ± 0.01	498 ± 25
Nondiabetic							
Sedentary	15	363 ± 8	218 ± 8	42.8 ± 0.4	14.5 ± 0.2	0.30 ± 0.03	463 ± 29
Exercised	6	358 ± 7	223 ± 11	43.0 ± 0.3	13.9 ± 0.1	0.33 ± 0.03	493 ± 22

Values are means ± SE; *n*, number of rats. *Nondiabetic groups are significantly different from diabetic groups ($P < 0.05$).

(DuPont, Wilmington, DE) at -70°C for 2–4 days. Plasma IGFBP-1 was determined by Western blot analysis. Briefly, plasma samples were separated on a 12.5% (wt/vol) SDS-polyacrylamide gel under nonreducing conditions, as previously described (38). Separated proteins were electroblotted onto nitrocellulose and blocked for 2 h at room temperature with Tris-buffered saline containing 1% nonfat dry milk. The membranes were then incubated with a 1:2,000 dilution of antiserum against rat IGFBP-1 at room temperature for 2 h. Antigen-antibody complexes were identified with goat anti-rabbit IgG conjugated with horseradish peroxidase (Sigma Chemical) and exposed to the enhanced chemiluminescence detection system (Amersham) for 1 min and to X-ray film for 10–30 s. Bands were scanned (Microtek ScanMaker IV) and quantitated using NIH Image 1.6 software. Representative samples from all experimental groups were electrophoresed on the same gel.

Nonesterified free fatty acids were determined enzymatically (NEFA-C kit, Wako Chemicals, Richmond, VA). This method is based on the acylation of CoA by the fatty acids in the presence of acyl-CoA synthase. The acyl-CoA produced is oxidized by acyl-CoA oxidase with the generation of hydrogen peroxide. The hydrogen peroxide in the presence of peroxidase permits the condensation of 3-methyl-*N*-ethyl-(β -hydroxyethyl)-aniline with 4-aminoantipyrine to form a colored compound, which is measured colorimetrically at 550 nm (13).

Statistical analysis. Statistical differences among groups were assessed using ANOVA. When significant *F* ratios were present, a Student-Newman-Keuls post hoc procedure was used to evaluate differences among means. A 0.05 level of confidence was chosen a priori. The number of rats in each group is indicated. Values are means ± SE.

RESULTS

Table 1 provides characteristics of the rats used in the study. Plasma glucose concentrations were similar between diabetic groups: range 507–608 and 508–600 mg/dl in exercised and sedentary rats, respectively. Nondiabetic rats had lower plasma glucose concentrations and greater body weights than the diabetic groups, but similar Hb and hematocrit values were found among all the groups. There were no significant differences in plasma corticosterone concentrations between the groups. Exercise, 16 h before assessment, did not change levels of plasma free fatty acids in nondiabetic or diabetic rats. However, free fatty acid levels were higher in diabetic than in nondiabetic rats ($P < 0.05$).

Figure 1 provides rates of protein synthesis for the various groups. No significant difference was found between rates of protein synthesis for sedentary and acutely exercised diabetic rats, and rates for both

groups were significantly ($P < 0.05$) lower than for nondiabetic groups. Rates of protein synthesis were higher in exercised nondiabetic rats than in sedentary rats. Figure 2 provides arterial plasma insulin concentrations at the time rates of protein synthesis were measured. Prior exercise did not alter insulin concentrations in diabetic or nondiabetic rats. Group means were markedly lower for severely diabetic than for nondiabetic rats.

Plasma IGF-I (Table 2) concentrations in severely diabetic groups were not different between exercised and sedentary rats but were significantly lower than in nondiabetic controls ($P < 0.05$). Muscle IGF-I (Table 2) was higher in the exercised severely diabetic than in sedentary rats (3.65 ± 0.7 vs. 2.32 ± 0.6 ng IGF-I/g muscle); however, this difference was not statistically significant ($P = 0.1$).

The large majority of IGF-I in the circulation is carried bound to one of at least six different high-affinity binding proteins. In the present study we determined the plasma concentration of two of these IGFBPs, IGFBP-1 and IGFBP-3. Diabetic rats had an ~10-fold elevation in the concentration of IGFBP-1 compared with nondiabetic rats (Table 2). Moreover, there was no detectable effect of exercise on nondiabetic or diabetic rats compared with their respective sedentary controls. In contrast, diabetic rats had a >50%

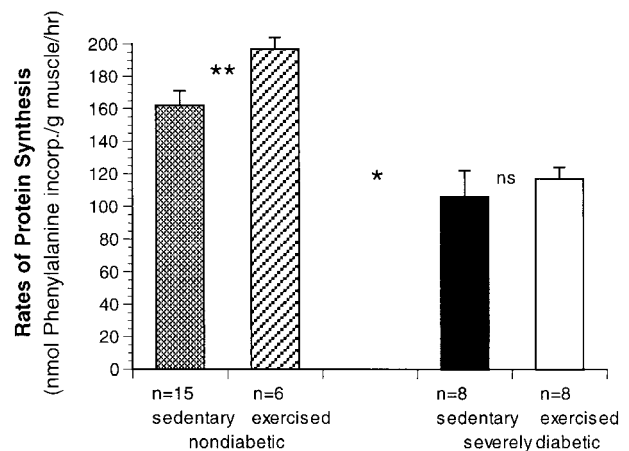


Fig. 1. Rates of protein synthesis for nondiabetic sedentary rats and severely diabetic rats that exercised or remained sedentary. Values are means ± SE. *Values for nondiabetic rats are significantly higher ($P < 0.05$) than for diabetic groups, which did not differ from each other [not significant (ns)]. **Values for nondiabetic exercised rats are significantly higher ($P < 0.05$) than for nondiabetic sedentary rats.

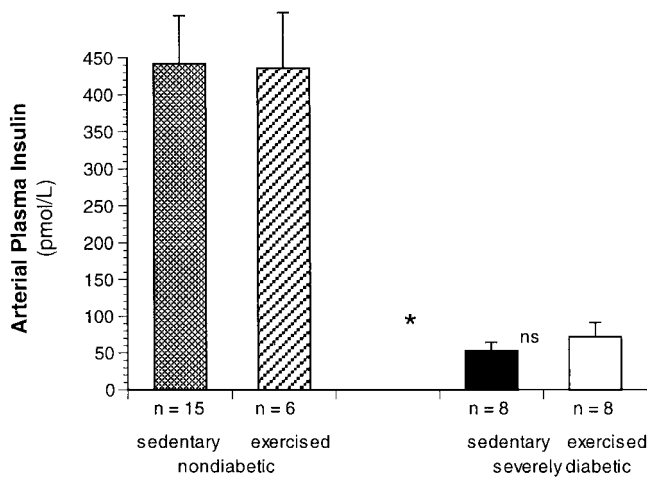


Fig. 2. Arterial plasma insulin concentrations for nondiabetic sedentary rats and severely diabetic rats that exercised or remained sedentary. Values are means \pm SE. *Concentrations for nondiabetic rats were significantly ($P < 0.05$) higher than for diabetic groups, which did not differ each other (ns = not significant).

decrease in the circulating concentration of IGFBP-3 compared with nondiabetic rats (Table 2). Again, there did not appear to be any significant effect of exercise on plasma IGFBP-3 levels in either group of animals.

Figure 3 provides data on 97 rats from the present and previous studies (16–18, 21, 22). Data are from previous publications and included rats that were moderately diabetic ($n = 29$), severely diabetic ($n = 19$), and nondiabetic ($n = 49$). For clarity of presentation, no distinction was made between exercised and sedentary rats, since several (18, 22) previous studies have shown that acute exercise does not alter arterial plasma insulin concentrations in nondiabetic rats. In one study, however, we observed lower plasma insulin concentrations in exercised than in sedentary moderately diabetic rats (17). Over a wide range ($\cong 80$ –600 pM) of insulin concentrations, there was no association between insulin concentrations and rates of protein synthesis. However, rates of protein synthesis were reduced when circulating insulin fell below ~ 80 pM. This value was determined as the intersection of two linear regression lines based on plasma insulin concentra-

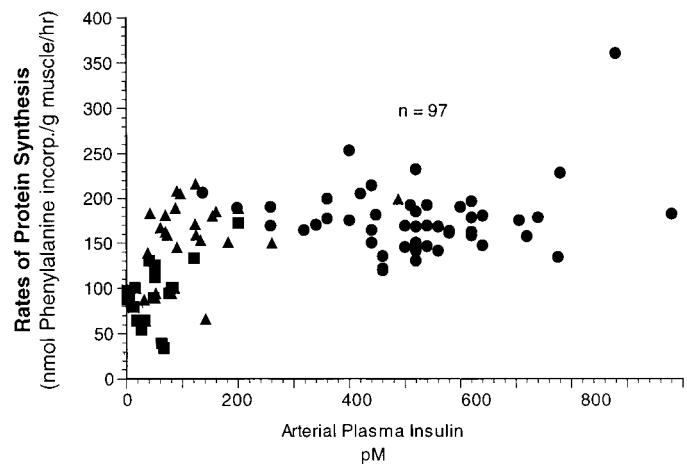


Fig. 3. Relationship between arterial plasma insulin and rates of protein synthesis for 97 rats. Data from present study and from previous studies are shown (16–18, 21, 22). All insulin samples were obtained from 5-h fasted rats immediately before determination of rates of protein synthesis. ●, Nondiabetic rats; ▲, moderately diabetic rats; ■, severely diabetic rats.

tions >120 pM (with the associated rates of protein synthesis) or <100 pM.

DISCUSSION

Elevations in rates of protein synthesis are consistently observed using not only our model (16–18, 21, 22) but also other models of resistance exercise (7, 8, 53, 54). The most important finding in this study is that rates of protein synthesis are not elevated after acute resistance exercise in severely diabetic rats. This lack of effect is in contrast to significant elevations in moderately diabetic rats after similar exercise (18). The model of resistance exercise used in the present study increases rates of protein synthesis 26–93% (16, 21, 22) in nondiabetic rats and 17–38% (9) in moderately diabetic rats. In the latter rats, 5-h fasted plasma glucose concentrations were 320–410 mg/dl and circulating insulin concentrations were about twice (17) those observed in the severely diabetic rats used in this study. The markedly reduced insulin concentrations in the present study may partially explain why an expected increase in rates of protein synthesis did not occur after exercise.

The amount of insulin required for normal anabolic responses after acute exercise is difficult to establish. The timing of the measurement of protein synthesis after exercise is also an important consideration, since shortly after (1 h) intense treadmill exercise, insulin does not augment exercise-induced changes in protein synthesis (3, 27). Data in Fig. 3 support those findings, but only from the perspective of high or elevated circulating insulin. When insulin drops below a critical concentration ($\cong 80$ pM in a 5-h fasted state), the inhibitory effect of hypoinsulinemia on the ability to maintain or increase rates of protein synthesis is marked. The finding that a low critical concentration of insulin is needed for an anabolic response after a stress is not incompatible with numerous studies in humans and rats showing that acute hyperinsulinemia does not

Table 2. Arterial plasma concentrations of IGF-I, IGFbps, and muscle concentrations of IGF-I for groups of rats

Group	<i>n</i>	IGF-I, ng/ml	IGFBP-1, AU	IGFBP-3, AU	Muscle IGF-I, ng/g muscle
Diabetic					
Sedentary	8	420 \pm 66†	4,238 \pm 526†	1,418 \pm 495†	2.32 \pm 0.6
Exercised	8	409 \pm 81†	2,306 \pm 504†	1,292 \pm 350†	3.65 \pm 0.7
Nondiabetic					
Sedentary	15	1,240 \pm 49*	360 \pm 76*	3,542 \pm 214*	ND
Exercised	6	1,143 \pm 60*	414 \pm 143*	4,172 \pm 501*	ND

Values are means \pm SE; *n*, number of rats. IGF-1, insulin-like growth factor I; IGFBP, IGF binding protein; ND, not determined; AU, arbitrary units. Within each variable, values with different symbols (*, †) are significantly different from each other ($P < 0.05$).

elevate protein synthesis when the organism is in a quiescent state (4, 28, 45, 58). Thus, above the critical concentration found in the present study, insulin is probably permissive, rather than regulatory, for protein synthesis.

The flooding-dose technique and the constant-infusion method, the two methods commonly employed to measure *in vivo* rates of protein synthesis, require assumptions and have potential limitations. The strengths and weaknesses of these two techniques have been reviewed (25, 48). Although these techniques typically provide reasonable agreement, discrepancies between them can exist. Davis et al. (11) showed in neonatal pigs that, 30 min after a flooding dose of phenylalanine as the radiolabeled precursor, there is an equilibrium of the phenylalanine-specific radioactivity between blood, phenylalanyl-tRNA precursor pool, and skeletal muscle tissue. Thus blood phenylalanine-specific radioactivity during the flooding-dose technique is an accurate estimate of the precursor pool of phenylalanyl-tRNA in skeletal muscle. This recent study supports the validity of using a flooding dose of phenylalanine to measure *in vivo* rates of protein synthesis in skeletal muscle. Although we acknowledge its potential limitations, we (16–18, 21, 22, 51) and many others (33, 42) have used the flooding-dose technique extensively to study changes in rates of protein synthesis in rats as a result of various perturbations.

The rats used in these studies were mature yet still growing. There are differences in insulin-mediated protein synthesis between mature and growing animals. Davis and co-workers (10, 55) showed that there is a curvilinear relationship between plasma insulin and skeletal muscle rates of protein synthesis in neonatal pigs and that this response declines with development. Studies in humans and rats demonstrate that protein synthesis in response to insulin is attenuated with aging. It is important to note that these studies were performed in nonstressed conditions. However, Fluckey et al. (21) showed that, with the addition of a resistance exercise stimulus, skeletal muscle protein synthesis remains insulin sensitive in old rats. The availability of insulin and its effects on skeletal muscle protein metabolism are thus relevant to young and old populations.

In addition to insulin, many other factors, including IGF-I, growth hormone, anabolic steroids, corticosterone, nutritional status, and amino acid availability, contribute to protein stability (34, 47, 50). We previously showed that food intake is not affected by the exercise model used in this study (21, 22). Yan et al. (56) reported that IGF-I immunoreactivity in tibialis anterior muscle increased at 4 days after eccentric exercise but not before this time in nondiabetic rats. Our previous work (18) supports this finding, since we found no significant change in muscle IGF-I 16 h after exercise in nondiabetic rats. These previous data were the reason we did not measure muscle IGF-I in nondiabetic rats in this study (Table 2). However, muscle IGF-I was significantly elevated in moderately diabetic rats in the previous study, suggesting that intramuscular IGF-I may play a compensatory role to facilitate an

appropriate anabolic response after resistance exercise in moderately hypoinsulinemic rats (18). Unlike mildly diabetic rats, data in Table 2 demonstrate that muscle IGF-I in severely diabetic rats was not significantly different as a result of exercise. Thus severe diabetes may hinder a compensatory role by muscle IGF-I under these conditions.

Although it is accepted that circulating levels of IGF-I are a reflection of growth hormone-mediated production by the liver, the regulation of nonhepatic IGF-I expression is less clear. In addition to acting in a classical endocrine manner, IGF-I is thought to have paracrine and autocrine functions as well. This is illustrated by the finding that in mice a significant increase in muscle IGF-I is not paralleled by a concomitant increase in plasma IGF-I (9). Regulation of IGF-I production is influenced by such regulators as hormonal levels, nutritional status, and numerous mitogenic and myogenic factors. Differential expression of the IGF-I gene as a result of alternative splicing or multiple transcription start sites allows for the tissue-specific regulation of IGF-I (49). Yang et al. (57) showed in skeletal muscle that there is a specific IGF-I transcript that is expressed after mechanical stretching. This could explain our finding of elevated intramuscular IGF-I in moderately diabetic rats after resistance exercise (18). However, neither nondiabetic nor severely diabetic rats exhibited this same effect. The reason for this is unclear, but perhaps insulin is performing a functional role in regulating muscle IGF-I production. This failure to increase muscle IGF-I, in addition to hypoinsulinemia, may be partially responsible for the inability of the severely diabetic rats to increase rates of protein synthesis after resistance exercise.

A pattern similar to that for muscle IGF-I existed for plasma IGF-I. The concentration of this factor increased with exercise in moderately diabetic rats (18); however, a diabetes-induced reduction in the circulating concentrations of this factor (50) was not overcome by exercise. In the present study, severely diabetic rats had very low plasma IGF-I concentrations, which were not changed by exercise. One interpretation of these results is that an anabolic response to resistance exercise is not possible when plasma IGF-I, insulin, and muscle IGF-I concentrations are extremely low.

The bioavailability and bioactivity of IGF-I can be influenced by the prevailing concentration of IGF-BPs (40). In the present study we examined the influence of diabetes and/or exercise on the relative concentration of two important IGF-BPs. First, IGF-BP-3 was assessed, because it carries the large majority (>80%) of IGF-I in the blood. Our results demonstrate a >50% reduction in plasma IGF-BP-3 in sedentary diabetic rats compared with sedentary nondiabetic rats. This diabetes-induced decrease in IGF-BP-3 has been previously reported (5). Furthermore, there was no effect of exercise in either group of animals. Previous studies have indicated that various types of exercise result in an increase (37) or no change (44) in plasma IGF-BP-3 levels. Differences in the type, duration, and/or intensity of exercise between these studies may explain differences in the IGF-BP-3 response to various exercise

stimuli. A smaller amount of IGF-I in the blood is also bound to IGFBP-1, which is a lower-molecular-weight binding protein thought to be responsible for the acute regulation of IGF-I bioavailability (40). As previously described by others (6), IGFBP-1 is markedly elevated by diabetes. This increase appears to be at least partly due to the severe hypoinsulinemia present in this condition (6). Again, however, there was no detectable effect of exercise on IGFBP-1. This lack of an exercise-induced change in IGFBP-1 is consistent with some studies (37) but differs from others, which demonstrate mild elevations (44) after exercise. As with IGFBP-3, the exact type, duration, and intensity of the exercise stimuli necessary to produce changes in one or more of the IGFBPs are unclear.

It is also important to note that most of the previous studies that demonstrate an effect on rates of protein of normalizing insulin from low to normal concentrations (19, 35, 41, 42, 46) have manipulated insulinemia for very short periods (hours or days). Rats in the present study were diabetic for ≥ 5 wk, and this prolonged hypoinsulinemia may alter the effectiveness of insulin for stimulating anabolism and have other adverse metabolic perturbations. Although a growth curve was not provided for the rats in this study, we previously showed that rats that are moderately diabetic for >10 wk still grow, albeit at a slightly reduced rate compared with nondiabetic rats (16). Likewise, the rats continue to grow during the 7-day period of weight lifting. Additional studies are needed to determine the importance of duration of diabetes on anabolic responses after exercise.

Summary. In contrast to nondiabetic and moderately diabetic rats, severely diabetic rats cannot elevate rates of protein synthesis after acute resistance exercise. Above a certain concentration of circulating insulin (≈ 80 pM in a 5-h fasted state), rates of protein synthesis are not associated with circulating insulin, even when those insulin concentrations are too low to maintain normal glycemia. In moderate but not severe diabetes, other regulators of protein synthesis, such as muscle IGF-I, are able to compensate for the hypoinsulinemia (or other untested factors) to allow appropriate anabolic responses. The results of this study suggest that there is a critical concentration threshold for insulin below which anabolic responses are negated. Future studies need to determine whether a minimum level of circulating insulin alone is sufficient to ablate anabolic responses or whether marked hypoinsulinemia must be coupled with reduced circulating or muscle IGF-I or other factors for this deficit to occur.

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