Effects of intensity of acute-resistance exercise on rates of protein synthesis in moderately diabetic rats

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Farrell, Peter A., Mark J. Fedele, Thomas C. Vary, Scot R. Kimball, and Leonard S. Jefferson. Effects of intensity of acute-resistance exercise on rates of protein synthesis in moderately diabetic rats. J. Appl. Physiol. 85(6): 2291-2297, 1998.—These studies determined whether increases in rates of protein synthesis observed in skeletal muscle after moderate or severe acute-resistance exercise were blunted by insulinopenia. Rats (n = 6-9 per group) were made insulin deficient by partial pancreatectomy or remained nondiabetic. Groups either remained sedentary or performed acute-resistance exercise 16 h before rates of protein synthesis were measured in vivo. Exercise required 50 repetitions of standing on the hindlimbs with either 0.6 g backpack wt/g body wt (moderate exercise) or 1.0 g backpack wt/g body wt (severe exercise). Insulin-deficient rats had a mean blood glucose concentration >15 mM and reduced insulin concentrations in the plasma. Rates of protein synthesis in gastrocnemius muscle were not different in all sedentary groups. The moderate-exercised nondiabetic group (192 ± 12 nmol phenylalanine incorporated·g muscle·h−1) and moderate-exercised diabetic group (215 ± 18) had significantly (P < 0.05, ANOVA) higher rates of protein synthesis than did respective sedentary groups. In contrast, diabetic rats that performed severe-resistance exercise had rates of protein synthesis (176 ± 12) that were not different (P > 0.05) from diabetic sedentary rats (170 ± 9), whereas nondiabetic rats that performed severe exercise had higher (212 ± 24) rates compared with nondiabetic sedentary rats (178 ± 10) P < 0.05. The present data in combination with previous studies (J. D. Fluckey, T. C. Vary, L. S. Jefferson, and P. A. Farrell. Am. J. Physiol. 270 (Endocrinol. Metab. 33): E313-E319, 1996) show that the amount of insulin required for an in vivo permissive effect of insulin on rates of protein synthesis can be quite low after moderate-intensity resistance exercise. However, severe exercise in combination with low insulin concentrations can ablate an anabolic response.

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CONTROLLERS OF ANABOLIC PATHWAYS must be upregulated after resistance exercise, since rates of protein synthesis are elevated at this time (3, 10, 40, 41, 43). Several studies show that insulin must be present for normal elevations in rates of protein synthesis to occur after moderate-intensity resistance exercise in rats (10, 11). These studies used both in vivo and in situ methods to establish this fact; but the interpretation of those studies is limited to comparisons of the effects of either

peptide chain initiation; insulinopenia; workloads

METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. Male Sprague-Dawley rats were used in all experiments and were housed in temperature- and humidity-
controlled holding facilities, with lights on at 0700 and off at 1900. Rats that were to be used as nondiabetic controls initially weighed 200–250 g, whereas rats that were to be made diabetic initially weighed between 100 and 125 g. Younger rats arrived in the laboratory about 1 mo before control rats, so that the two groups would weigh approximately the same at death. Diabetic rats were ~3 wk older than nondiabetic rats at death. The difference in initial weights between groups allowed for the 3-wk recovery period after surgery (described below). In preliminary studies using rats with initial weights of 120 g, we observed that nondiabetic rats grew at ~5.6 g/day, whereas moderately diabetic rats grew at a rate of 5.0 g/day. The number of rats in each group is included in Figs. 1 and 2 or in Table 1 presenting those data.

Partial pancreatectomy (PPX). These studies required the use of rats that were diabetic, but their glucose concentrations were not controlled by the administration of insulin on a daily basis. Although this can be accomplished by using nonlethal amounts of cytotoxic drugs (streptozotocin or alloxan), such drugs were not used, since their effects are not limited to the β-cell (37). The partial pancreatectomy procedure (12) was modified to include the use of older rats (110–140 g), as opposed to the weights (90–110 g) suggested by Folgia (12). We found that a larger percentage (60–80%) of the animals became diabetic when heavier rats were pancreatectomized. We also used a microcauterizer to eliminate some small pancreatic blood vessels to reduce bleeding. At the conclusion of surgery, rats were given ampicillin subcutaneously (5 mg/100 g body wt) as an antimicrobial agent. Two weeks after PPX, a tail vein blood sample was obtained with the rat in the fed state for the determination of blood glucose, and rats that were not diabetic (~175 mg/dl) were eliminated from the study. As in our previous work (6), even though the same regions of the pancreas were removed (90% PPX), a wide range of hyperglycemia resulted after a 2-wk recovery period.

Sham controls. One-half of the nondiabetic rats were sham operated, so that nonspecific effects of surgery could be assessed. Sham-operated rats were anesthetized, a laparotomy was performed, and the pancreas was exposed and gently rubbed between the fingers of the surgeon, and then closed, and the rats were given ampicillin identically to PPX rats. Laparotomies were performed at least 3 wk before the determination of rates of protein synthesis. Rates of protein synthesis were similar between sham-operated and control rats; therefore, the data for these groups were combined.

Resistance exercise. Details of the exercise protocol and the modifications we have used previously have been described (9, 11). This model of resistance exercise was chosen because it closely mimics the leg squat as performed by humans, and the drive for muscle contraction begins in the brain. We realize that direct electrical stimulation of the lower limbs provides better control over the force production (4, 40, 42); however, those models are less similar to the lifting procedures performed by humans. It should also be noted that even in the direct muscle-stimulation models neither the absolute power nor the power relative to the maximal capacity of the muscle can be directly measured. This is especially true during submaximal contractions. Briefly, in the model of resistance exercise we used, rats were operantly conditioned to touch an illuminated bar low on a cage and then to stand and touch an illuminated bar high on the opposite wall of the cage to avoid an electrical foot shock (~1 mA, 60 Hz). Once the learning process was completed (3–4 sessions), a weighted vest was strapped over the scapula, and the rat was required to touch the high bar fifty times during one session. We defined “acute”-resistance exercise (moderate group) as four separate sessions with 1-day rest between sessions. The rats performed 50 repetitions with 0.2 (day 1), 0.4 (days 2 and 3), and 0.6 (day 4) g weighted vest/g body wt. Previous work had shown that a naive rat would not lift the 0.6 g/g body wt on the first day weights were applied to the vest. These 4 days of exercise do not result in changes in muscle weight (10). Rats that performed severe exercise (severe group) were familiarized and exercised on four sessions in an identical manner as the moderate group, with the exception that the weights were 0.4 (day 1), 0.6 (days 2 and 3), and 1.0 (day 4) g weighted vest/g body wt. Exercise sessions occurred in the dark in the late afternoon. Nonexercised animals were placed in the cages and shocked five times over a period of ~10 min to simulate some of the stress experienced by the lifters. Familiarization and the resistance-exercise protocols required ~3 wk, thus the diabetic rats were 5–6 wk postsurgery at the time of death.

Because of the large number of groups of rats (n = 8) and the fact that it was not possible to study all groups simultaneously, we chose to use multiple sedentary groups that matched each exercise group as closely as possible. The sedentary groups were studied simultaneously with exercised groups. Rats were exercised, and flooding-dose protocols (see below) were performed for the moderate-exercise groups (and sedentary groups) and for severely exercised groups (and sedentary groups) within a 5-mo period.

Rates of protein synthesis. All measurements of rates of protein synthesis occurred 16 h after the last bout of 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. Total time between the onset of anesthesia and completion of surgery was 13–19 min. Rats remained unconscious during the flooding-dose protocol used to measure rates of protein synthesis that immediately followed the insertion of catheters. One milliliter of arterial blood was taken for the determination of insulin and glucose. After cannulation, a flooding dose (13) of tritiated L-[2,3,4,5,6-3H]phenylalanine (F) (1 mCi/rat; Amersham Life Science, Arlington Heights, IL) in cold phenylalanine (150 mM; 1 ml/100 g body wt total volume) was injected 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. Muscles to be used for rates of protein synthesis were dropped into liquid nitrogen. Rates of protein synthesis were measured separately for each animal. Frozen muscles were stored at −70°C until F incorporation into trichloroacetic acid-precipitable protein was measured by using pulverized tissue. Beta radiation in the protein precipitate was measured by using liquid scintillation counting with appropriate correction for volume, color, and particle quenching. Plasma-specific radioactivity of phenylalanine was analyzed after dabsylation (5) of the amino acid and quantification of the amount of phenylalanine by using HPLC. Radioactivity in the F chromatographic peak was measured by liquid scintillation counting with appropriate correction for quench. The specific radioactivity of plasma phenylalanine was used as the estimate of the precursor pool for phenylalanine (13). Protein determinations were made with the biuret method, and those values were used in the calculation of rates of protein synthesis. The amount of phenylalanine incorporated into muscle protein was calculated by using the method of Garlick et al. (13).

Plasma insulin concentrations were assayed by using a double-antibody radioimmunossay that is specific for rat insulin (6). Rat insulin standards were graciously provided by...
Eli Lilly (Indianapolis, IN.) Glucose was measured by the glucose oxidase method using a Yellow Springs Instruments model 23AM glucose analyzer.

Statistics. Because of the large number of exercised groups in this study and the fact that not all groups could be represented during each day of tissue procurement, two separate groups of sedentary normoinsulinemic and PPIX sedentary rats were included at the time of death for the moderate- and severe-exercise studies. This allowed us to euthanize equal numbers of exercised and sedentary rats on each tissue-procurement day over a 5-mo period. Data were analyzed by using a one-way analysis of variance with fixed and random effects of the randomized block design. The MIXED Procedure of SAS was used, and each variable was tested separately with all groups included in each analysis. This allowed comparisons based on both the effects of exercise and the effects of diabetes, separately and combined. When a significant F-ratio was calculated, a Student-Newman-Kuels procedure was used to locate significant mean differences. A P < 0.05 was chosen as a statistically significant difference. Physical and physiological data are reported as means ± SD, whereas variables in graphs are presented as means ± SE.

RESULTS

Physical and physiological characteristics of the eight groups of rats are provided in Table 1. The PPIX procedure was successful in creating diabetic animals; however, the degree of diabetes was mild in comparison to chemical diabetes. By design, the diabetic rats were ~3 wk older than nondiabetic rats at the time of determination of rates of protein synthesis. This allowed comparisons of groups with approximately the same body weights. Body weights were statistically similar for all groups; however, for all comparisons, diabetic rats weighed slightly less than nondiabetic rats. In general, the diabetic rats in the moderate-exercise groups were more diabetic (and had higher insulin concentrations, as shown below) compared with diabetic rats in the severe-exercise groups, but the differences in glucose were not significant between diabetic rats that performed severe vs. moderate exercise.

All rats had little difficulty completing 50 repetitions with 0.6 g backpack wt/g body wt. Rats had greater difficulty completing the severe exercise. Two diabetic and one control rat could not complete the 50 repetitions with 1.0 g backpack wt/g body wt. All rats completed at least 35 repetitions on the day before measurements of rates of protein synthesis. Our subjective evaluation was that the diabetic rats had greater difficulty with the heavier weight compared with nondiabetic rats.

Insulin concentrations in arterial plasma samples taken immediately before measurement of rates of protein synthesis were provided in Fig. 1. As expected, the diabetic groups in both the moderate- and severe-exercise studies had significantly lower circulating insulin concentrations compared with nondiabetic rats. Insulin concentrations were similar in the nondiabetic moderate-exercise group compared with the nondiabetic sedentary group. In contrast, the mean insulin concentration for diabetic rats that performed moderate exercise was lower (P < 0.05) than that for the diabetic sedentary rats. In the severely exercised groups, nondiabetic rats had significantly higher insulin concentrations compared with sedentary rats. Insulin concentrations in the diabetic rats that performed severe exercise were lower than those in sedentary rats; however, this difference did not reach statistical significance (P = 0.07).

Rates of protein synthesis for the eight groups that performed moderate- and severe-resistance exercise are provided in Fig. 2. Rates of protein synthesis were similar for all sedentary groups regardless of diabetic status. Groups that performed moderate-resistance exercise had higher synthesis rates for both diabetic (32%) and nondiabetic rats (26%). In contrast, rates of protein synthesis in similar groups that performed severe-resistance exercise did not produce similar results (Fig 2). Rates of protein synthesis for severely exercised nondiabetic rats were higher than for sedentary nondiabetic rats, but the increase on a percent basis (15%) was not as high as that observed during moderate exercise. Finally, rates of protein synthesis in diabetic rats that performed severe exercise were similar (4%) to rates found in sedentary diabetic rats.

Mean protein concentrations in gastrocnemius muscle ranged between 182 ± 26 and 214 ± 19 mg protein/g wet wt of muscle for all groups. None of the groups differed significantly from any other group (P > 0.17).

DISCUSSION

The present data help to clarify a role for insulin in the in vivo regulation of protein synthesis. Jefferson et al. (18) demonstrated that perfusion of skeletal muscle for >1 h with medium deficient in insulin induced a reduction in rates of protein synthesis that was reversed when insulin was added to the perfusion medium. Fluckey et al. (10) extended these observations to conditions when a physiological stress was present and the hindlimb perfusion was <30 min. Using a hindlimb-perfusion system (10, 11), we demonstrated that, when insulin was added to a perfusion medium, rates of protein synthesis were higher after moderate-resistance exercise than in sedentary controls. When insulin
was omitted from the perfusion medium, postexercise elevations in rates of protein synthesis were absent. Furthermore, the presence or absence of insulin did not alter rates of protein synthesis in muscle from nonexercised rats. These studies demonstrated that some insulin [or, perhaps, other compensatory growth factors (23)] must be present for normal anabolic responses to occur after moderate-resistance exercise. With these studies in mind, we expected a blunted elevation in rates of protein synthesis in insulin-deficient rats after both moderate and severe exercise. This was not the case, because in vivo rates of protein synthesis were higher after moderate-resistance exercise compared with sedentary rats that were either normoinsulinemic or insulin deficient.

Based on several previous reports (7, 16, 17), we also expected rates of protein synthesis in gastrocnemius to be lower in diabetic rats in a nonstressed state. This did not occur either (Fig. 2). These in vivo observations do not necessarily conflict with previous reports (1, 7, 19, 24, 25, 33) because of differences between our diabetic rats and those used in previous work, in terms of the severity and duration of diabetes. We used a PPX procedure that differs from chemical diabetes induction in that the rat can still produce some insulin. Previous work demonstrated reduced rates of protein synthesis in skeletal muscle within days after streptozotocin- or alloxan-induced diabetes. Such alloxan-induced decrements in protein synthesis were quickly (within 2 h) reversed by providing the rats with insulin. In those studies (1, 25), however, severe hypoinsulinemia was present such that the resulting hyperglycemia was much higher than that reported in this study. Also, rats in previous studies did not gain weight in the 2–4 days before death, and this was a criterion for documenting a diabetic state. The rats used in the present study were

Fig. 1. Arterial plasma insulin concentrations from blood samples taken immediately before determination of rates of protein synthesis. Rats were in the 5-h fasted condition. Values are means ± SE for n = 6–9 rats/group. sed, Sedentary; exerc, exercised. *Significant difference between exercised and sedentary groups, P < 0.05. **Values for diabetic rats were significantly lower than those for nondiabetic rats, P < 0.05. NS, not significant.

Fig. 2. Rates of protein synthesis in gastrocnemius for all groups of rats, n = 6–9 rats/group. Values are means ± SE. *Significant difference between exercised and sedentary groups, P < 0.05.
insulin deficient but still had measurable insulin levels and, consequently, were able to avoid excessive hyperglycemia. Also, our diabetic rats grew at a slightly reduced rate compared with nondiabetic rats. In previous literature, rats were diabetic for 2–7 days; however, our rats were diabetic for ~5 wk. While diabetic, our rats also did not display signs of major ill health, such as being dehydrated or having excessive concentrations of free fatty acids (data not shown) in the plasma. When combined, these facts suggest that insulin regulation of protein synthesis in long-term moderate diabetes may be different from the abnormal regulation reported previously (1, 7, 16, 18, 19, 24, 25, 31, 32) because of the total absence of insulin and consequent severity of diabetes.

In contrast to moderate exercise, however, our original hypothesis was confirmed in the case of severe exercise. Rates of protein synthesis were significantly higher after severe-resistance exercise in nondiabetic rats; however, the percent increase was about one-half of that observed after moderate exercise. Diabetic rats that performed severe exercise, however, had no such elevation in rates of protein synthesis compared with sedentary diabetic rats. This lack of an anabolic response could be due to the severe hypoinsulinemia in the diabetic/severe-exercise group. Insulin concentrations in this group just before the rates of protein synthesis were measured were only one-tenth those observed in the nondiabetic sedentary group and about one-half those observed in the diabetic sedentary group. To our knowledge, there are no previous studies that have determined whether severe exercise alters pancreatic function in hypoinsulinemic humans or rats. The markedly lower insulinemia in severely exercised diabetic rats probably contributed to an inability to elevate rates of protein synthesis.

Nondiabetic rats had higher rates of protein synthesis after severe exercise; however, the increase (15%) was lower relative to that observed after moderate exercise (26%). Wong and Booth (40) reported higher rates of protein synthesis in gastrocnemius muscle of rats 12–17 h after acute nonvoluntary resistance exercise only when the frequency of contractions was high. Their three different protocols were characterized as high frequency with either low or high resistance or as moderate frequency and moderate resistance. Wong and Booth’s data in nondiabetic rats suggest that rates of protein synthesis should have been higher in our study after the severe exercise compared with those after moderate exercise. This did not occur in either the nondiabetic or diabetic rats, since the relative increase in rates of protein synthesis was lower after severe vs. moderate exercise. It is difficult to compare our data, which used a whole body movement vs. direct electrical stimulation of the hindlimb, because the pattern of muscle activation is probably different between the two models. It is probable that all motor units are activated in the model used by Wong and Booth, whereas our model requires selective activation of those motor units needed to perform the task. We also note that the referenced study demonstrated that protein synthesis was consistently elevated after exercise when the contractions were at a high frequency (192 contractions/80 min total time, 2.4 contractions/min). Interestingly, those repetitions per minute were remarkably similar to our model (50 repetitions in ~25 min, 2.0 repetitions/min).

The flooding-dose technique as well as the constant-infusion technique have recognized limitations for measuring rates of protein synthesis, especially when the actual aminoacyl trNA precursor pool is estimated rather than measured directly. The strengths and weaknesses of these techniques have been reviewed (14, 35). Whereas the limitations of these techniques are recognized, the flooding-dose technique has been used in many studies that have compared rates of protein synthesis between diabetic and nondiabetic animals (1, 2, 20, 22, 31, 32; see Refs. 17 and 28 for review).

Insulin is a partial regulator of transmembrane amino acid transport; Jefferson et al. (20) used a hindlimb-perfusion technique to show that the effects of insulin in stimulating protein synthesis were not due to an effect of this hormone on amino acid availability in muscle cells. Also, the effect of insulin on system A-mediated amino acid transport is not rapid (26), and a differential transport during the flooding-dose period (10 min) seems an unlikely confounding variable. Also, we found no difference in basal rates of protein synthesis based on diabetic status; thus, if insulin deficiency caused a marked inhibition of amino acid transport, it was not evident under basal conditions. Therefore, a blunted ability to elevate rates of protein synthesis after severe-resistance exercise is probably not due to reduced amino acid transport.

Mechanisms that could explain blunted elevations in rates of protein synthesis are not clear but may center on insulin’s role in regulating several of the first steps in translation of mRNA (i.e., peptide chain initiation). Among other effects, severe insulin deficiency reduces the activity of eukaryotic initiation factor (eIF) 2B (22) and eIF2 (16), reduces the total skeletal muscle RNA (25), and results in an increased binding of eIF4E binding protein (phosphorylated heat- and acid-stable protein regulated by insulin) to eIF4E (27).

Through these and other pathways, hypoinsulinemia could blunt increases in mRNA translation after heavy-resistance exercise. Studies conducted in vivo that have assessed the degree of insulin deficiency needed to evoke such decrements in peptide chain initiation have not been reported. Our data show that the combination of a severe perturbation, such as heavy-resistance exercise, coupled to moderate insulin deficiency is sufficient to inhibit the elevations in rates of protein synthesis routinely observed in nondiabetic rats. Further studies are needed to assess the regulation of these factors as well as the involvement of other hormones that may account for the blunted anabolic response we observed (39). Among many others, such regulators as insulin-like growth factor I (21), thyroid hormones (23), or corticosterone (34) need to be considered.
The main focus of this study was insulin action rather than insulin secretion; however, the data warrant some discussion of the insulinaemia observed in the different groups. The effects of acute exercise on insulin secretion and availability to muscle have not been studied as extensively during resistance exercise as during endurance exercise. In humans, there is some suggestion that, although circulating concentrations of insulin are not altered by resistance exercise, concentrations of C peptide during an oral glucose tolerance test after resistance exercise are slightly higher (not significant) compared with resting conditions (8). We followed this suggestion of enhanced pancreatic insulin secretion after resistance exercise with a study in which we documented greater arginine-stimulated insulin secretion from pancreatic islets of rats that had performed resistance exercise, compared with sedentary rats. This postexercise effect was not observed when islets were stimulated by glucose (9). Thus the effects of prior resistance exercise on pancreatic secretion may depend on the nature of stimulus applied to the β-cell. In the present study, nondiabetic rats that performed severe exercise had significantly higher arterial plasma insulin in the 5-h fasted condition compared with nonexercised rats. The data for severe exercise support islet-level observations; however, insulin concentrations after moderate exercise were similar to those observed in nonexercised nondiabetic rats. Insulin concentrations in diabetic rats were lower after both moderate and severe exercise; however, the mean differences were significant only for the moderate-exercise condition. Diabetic rats that performed the more difficult exercise had lower insulin concentrations than did sedentary rats; however, this difference was not statistically significant. Because of the cross-sectional nature of our design, we do not know whether the lower insulin concentrations in severely exercised diabetic rats were the result of the last bout of exercise or whether they represented the normal state of insulinaemia for this group. These observations suggest, however, that pancreatic adaptations to acute resistance exercise may depend on the functional mass of the pancreas, the severity of the physiological perturbation, and the nature of the stimulus used to activate β-cells.

These in vivo studies also help advance the concept that insulin-related decrements in the ability to control glucose can occur in the absence of major decrements in the regulation of protein synthesis. The pleotropic actions of insulin have been reviewed recently (36), and it is clear that specific insulin-regulatable intracellular pathways exist for glucose transport and protein synthesis. We suggest that the use of a severe perturbation such as resistance exercise is appropriate for future studies that seek to define critical regulators in these pathways. Had we studied only resting animals, we would not have observed a functional decrement associated with insulinoopenia.

In summary, mildly diabetic rats had higher rates of protein synthesis after moderate-resistance exercise compared with sedentary diabetic rats. This shows that while the presence of insulin is required for a normal postexercise elevation in rates of protein synthesis, the amount of insulin needed for this permissive effect can be very small. During severe-resistance exercise, normoinsulinemic rats again had higher rates of protein synthesis (albeit reduced relative to moderate exercise); however, diabetic rats did not have higher synthesis rates. Thus the permissive effect of insulin in allowing elevations of anabolic pathways can be nullified by a severe perturbation such as severe-resistance exercise. These initial studies in rats suggest that carefully controlled studies involving humans with insulin deficiency performing moderate-resistance exercise could be considered an aid to help such persons avoid losses of muscle mass associated with long-term, poorly controlled type 1 diabetes mellitus.

We thank Marlin Druckenmiller, Fred Weyandt, Doug Johnson, Jen West, Mike Abraham, Jayee Cestyak, and John Miller for their superb technical efforts.

These studies were supported by the National Institutes of Health Grants AR-43127 (to P. A. Farrell), GM-39277 (to T. C. Vary), and DK-15658 (to L. S. Jefferson).

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Received 9 March 1998; accepted in final form 10 August 1998.

REFERENCES


