Hypertrophy of skeletal muscle in diabetic rats in response to chronic resistance exercise

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Farrell, Peter A., Mark J. Fedele, Jazmir Hernandez, James D. Fluckey, John L. Miller III, Charles H. Lang, Thomas C. Vary, Scot R. Kimball, and Leonard S. Jefferson. Hypertrophy of skeletal muscle in diabetic rats in response to chronic resistance exercise. J. Appl. Physiol. 87(3): 1075–1082, 1999.—This study had the following objectives: 1) to determine whether diabetic rats could increase muscle mass due to a physiological manipulation (chronic resistance exercise), 2) to determine whether exercise training status modifies the effect of the last bout of exercise on elevations in rates of protein synthesis, and 3) to determine whether chronic resistance exercise alters basal glycemia. Groups consisted of diabetic or nondiabetic rats that performed progressive resistance exercise for 8 wk, performed acute resistance exercise, or remained sedentary. Arterial plasma insulin in diabetic groups was reduced by about one-half (P < 0.05) compared with nondiabetic groups. Soleus and gastrocnemius-plantaris complex muscle wet weights were lower because of diabetes, but in response to chronic exercise these muscles hypertrophied in diabetic (0.028 ± 0.003 vs. 0.032 ± 0.0015 g/cm for sedentary vs. exercised soleus and 0.42 ± 0.068 vs. 0.53 ± 0.041 g/cm for sedentary vs. exercised gastrocnemius-plantaris, both P < 0.05) but not in nondiabetic (0.041 ± 0.0026 vs. 0.042 ± 0.003 g/cm for sedentary vs. exercised soleus and 0.72 ± 0.015 vs. 0.69 ± 0.013 g/cm for sedentary vs. exercised gastrocnemius-plantaris) rats when muscle weight was expressed relative to tibial length or body weight (data not shown). Another group of diabetic rats that lifted heavier weights showed muscle hypertrophy. Rates of protein synthesis were higher in red gastrocnemius in chronically exercised than in sedentary diabetic rats when muscle weight was expressed relative to tibial length or body weight (data not shown). These data suggest but do not prove that muscle mass increased in these muscles due to a physiological manipulation (chronic resistance exercise), and the balance between these physiological processes determines muscle mass. Previous studies have demonstrated that a normal increase in rates of protein synthesis measured in situ after acute resistance exercise does not occur when insulin is omitted from the medium perfusing the hindlimb (11). We recently reported that moderately diabetic and nondiabetic rats can increase rates of protein synthesis after acute resistance exercise to a similar degree as long as the weight lifted is not excessive (6). Circulating insulin-like growth factor I (IGF-I) may act in a compensatory manner in diabetic rats after exercise, in that both muscle and circulating IGF-I were higher after acute exercise in diabetic but not nondiabetic rats (7).

Reduced muscle mass is a frequently observed characteristic of humans with severe insulin deficiency. The overall goal of the present study was to determine whether moderately diabetic rats could increase muscle mass in response to chronic resistance exercise.

Goldberg (16) demonstrated that young alloxan-diabetic rats could increase the mass of soleus and plantaris muscles in response to the nonphysiological ablation of the gastrocnemius. This elevation in muscle mass was observed 6 days after the withdrawal of exogenous insulin, and thus the effects of long-term diabetes with inherent complications could not be assessed. Muscle hypertrophy in response to a physiological stimulus in adult rats with long-term diabetes has not been reported.

Many endurance exercise training studies using humans or rats with insulin deficiency have been reported (17, 18, 20, 24, 25), but only two studies have reported the effects of resistance exercise on muscle strength. In the study of Mandroukas et al. (27), patients with insulin-dependent diabetes mellitus (type I diabetes) performed aerobic exercises and intervals of static and dynamic training of the muscles. They found significant increases in isokinetic torque after 20 wk of training as well as increases in the muscle fiber area for type II (primarily, type IIa) fibers and the mean fiber areas when all fibers were combined. These data suggest but do not prove that muscle mass increased in these patients with type I diabetes. Durak et al. (4) also showed that patients with type I diabetes can significantly increase strength after 30 sessions (10 wk) of resistance exercise. This also should translate into increased muscle mass; however, total body weight and the percentage of the body occupied by fat did not
change in these patients. By subtraction, lean body mass (with the assumption of a 3-compartment model with no change in total body water) did not change significantly. No studies have documented the ability of resistance exercise to increase muscle mass in diabetic humans or rats. Because Goldberg's study (16) used a nonphysiological manipulation to increase muscle mass over a few days and the human studies (4, 27) could not measure total muscle mass, we used an animal model that allowed us to accurately measure changes in muscle mass due to chronic resistance exercise. In the two human studies referenced, significant effects of resistance exercise on basal glycemia were found in one (4) but not in the other (27).

Another area of uncertainty, irrespective of insulin status, is whether the magnitude of increases in rates of protein synthesis that are observed after acute resistance exercise in untrained subjects is maintained as the muscle becomes trained by repeated sessions of resistance exercise. The present study was conducted to determine 1) whether moderately diabetic rats could increase muscle mass in response to chronic resistance exercise, 2) whether chronic resistance exercise alters basal glycemia in diabetic rats, and 3) whether elevations in rates of protein synthesis after exercise are diminished as rats train.

**METHODS**

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Pennsylvania State University. Male Sprague-Dawley rats 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 (PMI Feeds 5001) that contained 24% protein, 12% fat, 50% carbohydrate, 7% ash, 6% fiber, and vitamins.

Partial pancreatectomy. Diabetic rats in which glucose concentrations were not controlled by daily exogenous insulin were required for this study. We used a partial pancreatectomy (12) model to meet this need. Sterile conditions were maintained throughout the surgery. Rats were anesthetized using methoxyflurane and were kept on a heated surgical pad. Pancreatic tissue from the spleenic, duodenal, and pyloric regions was removed using sterile cotton Q-tips, with care taken to leave major blood vessels intact. Pancreatic tissue between the bile duct and the duodenum was not removed, since this approximates 10% of the original total pancreatic tissue. At the conclusion of surgery, rats were given ampicillin (5 mg/100 g body wt sc) 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, and rats that were not diabetic (<275 mg/dl) were eliminated from the study. In previous studies (5, 6) we observed reduced rates of somatic growth after partial pancreatectomy. This reduced growth rate is also expressed as reduced muscle growth; thus this model allowed us to determine whether chronic resistance exercise could ameliorate this diabetes-induced condition. Within 1 wk of verification of a diabetic status, diabetic and nondiabetic rats were randomly assigned to sedentary or exercised groups, with the latter immediately beginning resistance exercise training.

Chronic exercise. Details of the exercise protocol have been previously described (9). 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 (<3 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 exercise session. The rats performed 50 repetitions on each exercise day with three sessions per week over the 8-wk period. The average amounts of weight lifted for diabetic and nondiabetic groups are provided in Fig. 1. 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 last week of exercise training and were given five electric shocks to simulate some of the stress experienced by the exercised groups. One of these control (for shock) sessions occurred 16 h before the determination of rates of protein synthesis.

Additional groups of resistance-trained nondiabetic rats were studied to determine whether hypertrophy would occur if the rats lifted even more weight than shown in Fig. 1. The weight on the backpack was increased over 6 wk, with the final weight being ~1 g/g body wt. A separate control group was included for this comparison. None of the nondiabetic groups were sham operated, because it had been demonstrated previously (6) that rates of protein synthesis are not different between sham-operated and control rats that performed acute exercise or remained inactive.

Resistance exercise. Two additional groups of rats were studied simultaneously with the chronically trained groups so that comparisons could be made between the effects of acute exercise on rates of protein synthesis in trained vs. untrained rats. One group was diabetic and the other nondiabetic. Rats (both groups) were about 3 wk younger than the groups used for the chronic studies. Acute resistance exercise consisted of four separate exercise sessions with 1 day of rest between each session. On day 1, rats performed 50 repetitions with 0.2 g/g body wt. On days 2 and 3 they lifted 0.4 g/g body wt, and on the last session they lifted 0.6 g/g body wt for 50 repetitions. Rates of protein synthesis for red gastrocnemius muscle only were assessed 16 h after the last bout of acute exercise.

Rates of protein synthesis. All measurements of rates of protein synthesis occurred 16 h after the last bout of resistance exercise. Food was withdrawn from the rats during the last 5 h of this 16-h period. Rats were anesthetized with...
was chosen a priori. Because we could not make multiple measures of rates of protein synthesis on the same animal, the statistical analysis represents a comparison between means for different groups. Changes in plasma glucose with exercise training were analyzed using repeated-measures ANOVA. Values are means ± SE or SD as appropriate.

RESULTS

All rats were able to complete the lifting protocol, and the weights lifted on a weekly basis are shown in Fig. 1. Pilot studies had demonstrated that naive diabetic rats needed slightly more time to adjust to the weighted packs than nondiabetic rats. Therefore, we started the training for diabetic rats with slightly less weight. The initial weights were chosen on an empirical basis. During weeks 5–7 of training, diabetic and nondiabetic rats lifted the same weight relative to body weight. Some diabetic rats were more difficult to motivate during the last week of training; therefore, we reduced the required weight that was lifted 50 times each session. The physical and physiological characteristics of the rats are provided in Table 1.

Age-matched diabetic rats weighed less than nondiabetic rats. The rats used for the acute exercise studies were ~3 wk younger than rats in the trained groups. Additional data on body size provided in Fig. 2 show that the average weight of selected groups increased

Table 1. Physical and physiological characteristics of the groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Plasma Glucose, mg/dl</th>
<th>Insulin, pM</th>
<th>Hct, %</th>
<th>Hb, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic sedentary</td>
<td>8</td>
<td>469 ± 21*</td>
<td>227 ± 12*</td>
<td>483 ± 22</td>
<td>43.8 ± 1.2</td>
<td>14.4 ± 0.4</td>
</tr>
<tr>
<td>Nondiabetic trained</td>
<td>8</td>
<td>433 ± 31*</td>
<td>221 ± 28*</td>
<td>462 ± 73</td>
<td>42.9 ± 2.3</td>
<td>14.3 ± 0.6</td>
</tr>
<tr>
<td>Nondiabetic acute exercise</td>
<td>7</td>
<td>362 ± 19*</td>
<td>168 ± 24*</td>
<td>384 ± 62</td>
<td>43.0 ± 0.8</td>
<td>14.7 ± 0.9</td>
</tr>
<tr>
<td>Diabetic sedentary</td>
<td>9</td>
<td>328 ± 99*</td>
<td>128 ± 51†</td>
<td>212 ± 151†</td>
<td>44.1 ± 2.4</td>
<td>14.8 ± 0.6</td>
</tr>
<tr>
<td>Diabetic trained</td>
<td>7</td>
<td>363 ± 64*</td>
<td>458 ± 57</td>
<td>181 ± 40†</td>
<td>46.4 ± 2.5</td>
<td>15.2 ± 0.5</td>
</tr>
<tr>
<td>Diabetic acute exercise</td>
<td>10</td>
<td>301 ± 31†</td>
<td>385 ± 36</td>
<td>211 ± 41†</td>
<td>46.4 ± 1.3</td>
<td>15.0 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. Hct, hematocrit; plasma glucose represents glucose concentration at time of measurement of rate of protein synthesis. *Body weight is significantly greater and plasma glucose significantly lower in nondiabetic groups than in respective diabetic groups, P < 0.05. †Body weight for acutely exercised diabetic group is lower than for respective nondiabetic group, but these rats were also younger than trained rats, P < 0.05. ‡Plasma insulin concentrations are significantly lower in diabetic groups than in respective nondiabetic groups, P < 0.05.
during the training period. Chronic resistance exercise caused a slower rate of weight gain in nondiabetic rats but a faster rate for diabetic rats.

Circulating insulin concentration in sedentary diabetic rats was about one-half ($P < 0.05$) that observed in nondiabetic rats, and hematocrit and Hb did not differ in any group. Circulating IGF-I concentrations are provided in Fig. 3. Circulating IGF-I in diabetic sedentary rats was less than one-half ($P < 0.05$) that in nondiabetic rats but was significantly higher in chronically exercised diabetic rats. Exercise training had no effect on circulating IGF-I in nondiabetic rats.

The effect of chronic resistance exercise on muscle mass is provided in Figs. 4 and 5. Diabetes resulted in a significant ($P < 0.05$) reduction in the mass of the gastrocnemius-plantaris complex as well as soleus when expressed in absolute terms (data not shown) and when expressed relative to body weight (e.g., gastrocnemius-plantaris, 4.8 ± 0.48 and 6.5 ± 0.11 g/kg body wt for diabetic sedentary and nondiabetic sedentary) and relative to the length of the tibia (Figs. 4 and 5). Mass (relative to body weight or tibial length) of the gastrocnemius-plantaris and soleus muscles was significantly greater ($P < 0.05$) in exercise-trained diabetic rats, whereas no effect of chronic exercise was observed on these muscles in nondiabetic rats. Protein concentration (mg/g muscle) was not affected by hypoinsulinemia or exercise status (Fig. 6). The increase in wet weight represented increases in muscle mass, since the ratio of dry weight to wet weight of gastrocnemius and soleus was not different between chronically exercised and sedentary rats (data not shown). Another two groups of nondiabetic rats were studied to determine whether lifting greater weights over a 6-wk period resulted in hypertrophy. In rats that lifted 1.0 g/g body wt, hypertrophy was significant ($P < 0.05$) for exercised vs. sedentary rats: 8.0 ± 0.3 vs. 6.9 ± 0.3 g/kg. Tibial lengths were not determined in these two groups.

Rates of protein synthesis for sedentary and chronically exercised rats in each of the muscles studied are provided in Fig. 7. ANOVA for sedentary vs. chronic groups (only) showed that rates of synthesis were higher in red gastrocnemius and soleus but not in superficial white gastrocnemius and extensor digitorum longus (EDL) for nondiabetic rats. Rates of
DISCUSSION

One of the most important findings from this study is that although diabetic rats have poor glucoregulation and markedly reduced skeletal muscle mass, they can increase that mass by means of a physiological stimulus. A second new finding is that the effect of an acute bout of resistance exercise on stimulating protein synthesis seems to diminish as rats become trained in that activity and that adaptation is observed in normoinsulinemic and hypoinsulinemic rats. Finally, chronic resistance exercise training was associated with lower basal plasma glucose, which was not observed in sedentary rats.

Pioneering work 30 years ago (16) demonstrated that alloxan-diabetic rats could increase plantaris and soleus muscle mass in response to synergist ablation of the gastrocnemius. This hypertrophy occurred, despite the fact that, during insulin withdrawal, rats failed to gain total body weight. Our data are consistent with this previous study but differ in that Goldberg (16) studied rats over a 6-day period, whereas our rats lifted weights for 8 wk, during which they were gaining body weight. Another important difference between the present study and that of Goldberg is the duration of diabetes. It is unlikely that the effects of chronic hyperglycemia were present in Goldberg’s study because of the short duration of diabetes; however, in the present study, rats were diabetic for ~10 wk, which is sufficient time for specific glycation of certain proteins (38) as well as for changes in microcirculatory regulation (13). Both of these pathological developments could affect protein stability. Our data advance the concept of a maintained ability to elevate skeletal muscle mass during moderate long-term hypoinsulinemia in response to a physiological rather than a nonphysiological stimulus. The positive results, if verified in studies on patients with type 1 diabetes, would have practical significance, since maintaining muscle mass is a concern for such patients.

When diabetic and nondiabetic rats trained with the same weight relative to body weight, the diabetic rats increased muscle mass, whereas nondiabetic rats did...
not. This was not due to an inability of nondiabetic rats to increase muscle mass, since when heavier weights were lifted, hypertrophy occurred in those rats. Thus mechanisms that result in accrued muscle mass seem to be activated at lower relative muscle strains in diabetic rats. This observation stimulates the question of whether muscles from long-term moderately diabetic muscle can develop the same tension as nondiabetic muscle. This question has not been addressed.

Increased muscle mass can occur only through a change in the balance between protein synthesis and degradation. Insulin is a known stimulator of synthesis (14, 21, 22, 32) and an inhibitor of proteolysis (31, 37, 40). Using the same acute resistance exercise protocol used in the present study, Fluckey et al. (11) demonstrated that when insulin was omitted from a medium perfusing a rat hindlimb, normal postexercise elevations in rates of protein synthesis were ablated. Thus some insulin or perhaps other hormones are needed for an appropriate anabolic response. Farrell et al. (6) demonstrated that moderately diabetic rats could increase in vivo rates of protein synthesis after moderate-intensity resistance exercise. Thus, although some insulin (or another hormone) must be present, the amount of insulin can be quite low. This maintained ability of diabetic rats to increase rates of protein synthesis after exercise does not necessarily translate into elevations of muscle mass since hypoinsulinemia accelerates proteolysis, and this could negate any elevations in synthesis. Figures 6 and 7 clearly show elevated rates of protein synthesis after exercise in acutely exercised and trained diabetic rats that lifted ~0.6 g/g body wt. Rates of proteolysis were not assessed in this study; however, other studies show that proteolysis increases after resistance exercise (1). The fact that muscle mass increased in diabetic rats suggests a greater effect of exercise on synthesis than on proteolysis.

Nondiabetic rats that lifted ~0.6 g/g body wt did not increase muscle mass; however, when the weight lifted was increased by about one-third, significant hypertrophy occurred. We did not have diabetic rats lift greater weights, since, in contrast to nondiabetic rats, such animals do not increase rates of protein synthesis after severe exercise, whereas nondiabetic rats (6) increase synthesis, albeit with a lesser elevation. These results suggest that, under conditions of muscle atrophy, mass can be partially restored by resistance exercise, but identical exercise does not alter muscle mass in nonatrophyed animals. These data also confirm that very low levels of insulin are adequate to allow an anabolic response. The importance of the availability of insulin relative to the intensity of muscle contraction required to stimulate hypertrophy is not clear.

Figure 6 shows that for all muscles, with the exception of superficial white gastrocnemius, rates of protein synthesis were lower in sedentary diabetic than in sedentary nondiabetic rats. The reductions are consistent with previous reports but were smaller than reductions of ~50% reported in severely diabetic rats (8, 32). The work of Plaim et al. (8) also demonstrated a greater effect of severe hypoinsulinemia on muscle composed primarily of fast-twitch fibers. Our data are not consistent with this observation, but again the rats used in this study were moderately diabetic and were diabetic for many weeks vs. a few days.

We have no explanation for the fact that rates of protein synthesis were higher in EDL of chronically exercised diabetic rats but not nondiabetic rats. We previously reported a lack of an exercise response in EDL in nondiabetic rats (10, 11) and believed that this was reasonable on the basis of a probable lack of recruitment of this dorsiflexor during the lifting protocol. It is possible that diabetic rats recruit EDL during exercise because of the significant reduction in the mass of other muscles in the lower limb.

The fact that synthesis can increase despite very low concentrations of insulin suggests that, in this model, insulin may play a permissive role and that other factors are involved in the regulation of synthesis. Just as contractions per se stimulate glucose uptake (30, 34), it may be possible that muscle contractions produce a biophysical environment in muscle cells that allows enhanced action of otherwise quiescent regulators. Some work suggests that IGF-I is activated by exercise (7, 43), and this protein has anabolic effects (41). Circulating IGF-I was reduced because of diabetes but was higher in the diabetic trained group. Such an elevation did not occur in the nondiabetic trained group. The complexity of the IGF-I system precludes speculation at this time on how this factor may act in a compensatory role when elevations in rates of protein synthesis are required but hypoinsulinemia exists.

The effects of training status on exercise-induced elevations of protein synthesis have not been systematically investigated. Toward the end of the training program, diabetic and nondiabetic rats lifted ~0.55- and 0.66-g weighted backpacks per gram of body weight, respectively, for 50 repetitions per session. Nontrained but acutely exercised rats lifted 0.6 g/g body wt, and the resultant rates of protein synthesis were higher than in trained rats. Rates of protein synthesis for the acutely exercised nondiabetic rats were ~192 nmol phenylalanine incorporated·g muscle~1·h~1, which is higher than the ~150 nmol phenylalanine incorporated·g muscle~1·h~1 that we reported for sedentary rats of a similar age (320 g) (6). Thus the fact that the acutely exercised nondiabetic rats were 3 wk younger than trained rats probably did not account for the higher rates of protein synthesis. A reduced response in trained rats is not unexpected, since it is compatible with a general principle of adaptation to stress. As one becomes accustomed to a stress, the physiological perturbation due to that stress is reduced. It is interesting that the same adaptation to training occurred in hypoinsulinemic rats.

Only a few studies have investigated the effects of resistance exercise on glucose regulation. Studies using nondiabetic populations (2, 28) show that this type of training increases insulin sensitivity for glucose uptake. Only in studies by Mandroukas et al. (27) and Durak et al. (4) have such measurements been made.
before and after chronic resistance exercise in humans with type I diabetes. Although Mandroukas et al. reported no change in glycemia, the reduction in glycemia we observed agrees with the data from Durak et al. Durak et al. also reported a decrease in HbA1c associated with training.

In contrast to resistance exercise, many (35, 36, 39), but not all (18, 19), studies show that chronic endurance exercise can reduce glycemia in diabetic rats. This is not the case in humans, however; in most studies in which type I diabetic humans performed endurance exercise training, no change has been reported in glycemia or HbA1c (33, 42, 45). Because of this species difference and the paucity of information regarding resistance exercise, we suggest that more attention could be paid to the ability of resistance exercise to help reduce glycemia in patients with type I diabetes.

In summary, moderately hypoinsulinemic rats are not only capable of increasing rates of protein synthesis after resistance exercise but also can also increase muscle mass when that exercise is repeated on a regular basis. There is an adaptation to such repeated exercise, such that the effects of the last bout of exercise in terms of elevations in protein synthesis are diminished. These results, in combination with previous reports (10, 11), provide a foundation for future studies that should assess the safety and risk–to–benefit ratio for patients with type I diabetes who wish to engage in resistance exercise.

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