Effects of endurance exercise-training on single-fiber contractile properties of insulin-treated streptozotocin-induced diabetic rats

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1School of Kinesiology, University of Minnesota, Minneapolis, Minnesota; 2Department of Physiological Sciences, School of Medicine, Universidad de Oriente, Edo Bolivar, Venezuela; and Departments of 3Physical Medicine and Rehabilitation, and 4Biochemistry, Molecular Biology, and Biophysics, University of Minnesota Medical School, Minneapolis, Minnesota

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Sanchez, Otto A., LeAnn M. Snow, Dawn A. Lowe, Robert C. Serfass, and LaDora V. Thompson. Effects of endurance exercise-training on single fiber contractile properties of insulin-treated streptozotocin-induced diabetic rats. J Appl Physiol 99: 472–478, 2005.—The purpose of this study was to characterize the contractile properties of individual skinned muscle fibers from insulin-treated streptozotocin-induced diabetic rats after an endurance exercise training program. We hypothesized that single-fiber contractile function would decrease in the diabetic sedentary rats and that endurance exercise would preserve the function. In the study, 28 rats were assigned to either a nondiabetic sedentary, a nondiabetic exercise, a diabetic sedentary, or a diabetic exercise group. Rats in the diabetic groups received subcutaneous intermediate-lasting insulin daily. The exercise-trained rats ran on a treadmill at a moderate intensity for 60 min, five times per week. After 12 wk, the extensor digitorum longus and soleus muscles were dissected. Single-fiber diameter, Ca2+-activated peak force, specific tension, activation threshold, and pCa50 as well as the myosin heavy chain isoform expression (MHC) were determined. We found that in MHC type II fibers from extensor digitorum longus muscle, diameters were significantly smaller from diabetic sedentary rats compared with nondiabetic sedentary rats (P < 0.001). Among the nondiabetic rats, fiber diameters were smaller with exercise (P = 0.038). The absolute force-generating capacity of single fibers was lower in muscles from diabetic rats. There was greater specific tension (force normalized to cross-sectional area) by fibers from the rats that followed an endurance exercise program compared with sedentary. From the results, we conclude that alterations in the properties of contractile proteins are not implicated in the decrease in strength associated with diabetes and that endurance-exercise training does not prevent or increase muscle weakness in diabetic rats.

endurance training; diabetes; atrophy; force; specific tension

PATIENTS WITH LONG-TERM Type 1 diabetes mellitus demonstrate a decrease in muscle strength at the ankle and knee joints (4, 6). The observed muscle weakness can, in part, be explained by muscle atrophy secondary to diabetic motor neuropathy (5). However, other cellular mechanisms may be involved in the altered muscle performance associated with diabetes (7). Investigations using a whole muscle preparation show Type 1 diabetes-induced alterations in muscle performance (33). Although skeletal muscle atrophy plays a role in the decline in muscle performance, alterations at the single fiber level are implied because the effects of diabetes on skeletal muscle strength are still evident after normalizing for changes in muscle volume and weight (12).

To further elucidate the mechanisms responsible for muscle weakness associated with the diabetic state, Stephenson et al. (43) investigated the effects of diabetes on single-fiber contractile properties, using the permeabilized-fiber preparation. Specifically, single-fiber specific tension (peak force/cross-sectional area) decreased by 25% after 4 mo of severe diabetes without insulin treatment. These findings suggest that the quality of the contractile proteins declines with uncontrolled diabetes. However, because all Type 1-diagnosed diabetic individuals receive insulin therapy, we studied permeabilized fibers from muscles of rats with and without insulin therapy. We found that despite insulin administration, single-fiber diameter and peak force from diabetic rats were reduced compared with fibers from healthy control rats (37). Interestingly, muscle fibers from the diabetic rats with insulin had significantly larger diameters and peak forces than fibers from the diabetic group without insulin (37). These results suggest that insulin administration without tight glucose control can only attenuate the progression of muscle atrophy and decline in strength, but it does not totally prevent diabetes-induced muscle atrophy or weakness.

Endurance exercise training is recommended for patients with diabetes mellitus because it improves glucose control and reduces other risk factors (1). In normal subjects, endurance-exercise training increased capillarity (24), decreased capillary basement membrane width (46), and improved vasodilation in skeletal muscle thereby improving blood flow (10), which is required for the delivery of amino acids to skeletal muscles and stimulation of protein synthesis (44). Furthermore, endurance exercise increased IGF-I (14, 18), which has the potential to maintain muscle mass by activating satellite cells (11) and decreasing protein breakdown (28). In contrast, there is evidence for endurance training-induced fiber atrophy in type II cells (21). Because endurance training has the potential to either preserve muscle mass or induce atrophy in healthy rats, it is not known which of these effects will predominate in an insulin-treated diabetic state.

To mimic the clinical conditions observed in Type 1 diabetic patients, it is necessary to administer insulin and perform the recommended exercise protocol. Therefore, the purpose of this study was to determine the effects of endurance exercise training on the contractile properties of permeabilized muscle fibers from streptozotocin (STZ)-induced diabetic rats that received insulin treatment. We hypothesized that single-fiber contractile function would be compromised in the diabetic
needed to achieve a specific percentage of maximal O2 consumption. The protocol was based on previous research where the speed and grade were 2.5 m/min until the last 2 wk when they ran at a speed of 27 m/min per day starting at a speed of 15 m/min and increased every 2 wk by 2.5 m/min until the last 2 wk when they ran at a speed of 27 m/min.

Methods

Animal Care

The University of Minnesota Institutional Animal Care and Use Committee approved all experimental procedures. Sprague-Dawley rats, 7 wk of age and weighing between 200 and 250 g (Charles River, Wilmington, MA), were used in this study (2 died of hypoglycemia). They were housed in a light- and temperature-controlled animal facility. Food and water were available ad libitum. Rats were randomly divided into four groups: nondiabetic sedentary (n = 7), nondiabetic exercise (n = 7), diabetic sedentary (n = 7), or a diabetic exercise (n = 7). Diabetes was induced with the intravenous injection of STZ (Sigma), 60 mg/kg of body weight in saline solution. Nondiabetic rats were injected only with saline solution. Diabetes was confirmed 2 days later when blood glucose concentration was at least 250 mg/dl for 2 consecutive days. Thereafter, blood glucose was measured three times per week for the following 12 wk using the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Palo Alto, CA). An intermediate-lasting (16–24 h) subcutaneous neutral protamine Hagedorn insulin was administered daily to the diabetic rats at the beginning of the active phase in the evening, according to a sliding scale on the basis of the latest blood glucose values. Insulin was given to mimic the clinical condition of patients but not to provide tight glucose control. Blood glucose was maintained at ∼300 mg/dl with this insulin treatment.

Endurance Exercise Training Program

Rats assigned to the exercise groups ran five times per week for 60 min/day starting at a speed of 15 m/min and increased every 2 wk by 2.5 m/min until the last 2 wk when they ran at a speed of 27 m/min. The incline was maintained at 10% throughout the study. This protocol was based on previous research where the speed and grade needed to achieve a specific percentage of maximal O2 consumption and lactate threshold were determined (8, 35). During the initial 2 wk, rats started running at a level just below their estimated lactate threshold, and at the end they were running at intensities above their initial estimated lactate threshold. To assess training status, blood lactates were determined at the beginning and at the end of the 12 wk of training on all rats including those that were sedentary. Blood lactate was measured using a YSI lactate analyzer (YSI 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH) before and after a 10-min run at a speed of 20 m/min and 10% grade. A heparinized micropipillary tube was used to collect 20 μl of blood by venipuncture through the tail vein, and the sample was stored in Triton X-100 and sodium fluoride until measurement. The blood extraction procedure took 6–8 s to perform; therefore, blood lactate values were close to the actual values during exercise.

Muscle Preparation

Rats were anesthetized with pentobarbital sodium (50 mg/kg of body weight ip), and the extensor digitorum longus (EDL) and soleus muscles from the right hindlimb were used for the permeabilized single muscle fiber preparation. The muscles from the left hindlimb were used for biochemical analysis. All muscles were dissected, excised, and weighed. Muscles used for biochemical analysis were frozen in liquid nitrogen and stored at −80°C. The gastrocnemius muscles, superficial region (white gastrocnemius), were used to assess citrate synthase activity (42). Muscles prepared for the permeabilized fiber preparation were placed on a dissecting dish in ice-cold relaxing solution to form bundles and then stored separately in vials, at −20°C in a permeabilizing solution containing 125 mM K-propionate, 2 mM EGTA, 4 mM ATP, 1 mM MgCl2, 20 mM imidazole, (pH 7.00), and 50% glycerol (vol/vol) and the protease inhibitor leupeptin (0.1%, final concentration) (3). From each of the two muscles (EDL and soleus), we formed four to five bundles, which contained several hundred fibers each. In each vial, we stored two to three bundles. Bundles were placed in permeabilizing solution for a duration of 2–28 days.

Single-Fiber Experimental Protocol

On the day of the experiment, one of the vials was transported on ice to the experiment room and a bundle was taken out of the vial and placed in a dissecting dish in ice-cold relaxing solution, where a single muscle fiber was teased from the bundle. Subsequently, the bundle was placed back into the vial and kept on ice. At the end of the experiment, the rest of the unused bundle was placed again in −20°C until the next experimental session.

A single muscle fiber segment measuring between 3 and 4 mm in length was dissected and transferred to an experimental chamber. The chamber was mounted to the stage of an inverted microscope. The chamber included a spring-mounted stainless steel plate insert with three wells containing activating or relaxing solutions; this setup allowed the fiber to be transferred from the well, which contained the relaxing solution to a well, which contained the activating solution. The bottom of the well was sealed with a glass coverslip, which allowed the mounted fiber to be transilluminated for viewing (3). One of the ends of the fiber was mounted on the output shaft of a force transducer (sensitivity 2 mV/μg; model 403 A, Cambridge Technology), and the other end was attached to a fixed shaft. Thus the length of the fiber ranged between 1.5 and 2.5 mm. The fiber segments were then submerged in a well with relaxing solution (pH 7.00), baseline parameters were determined, and subsequently the fiber segments were submerged in an activating solution to determine the force-generating capacity. Both the relaxing and activating solutions contained 7.0 mM EGTA, 5.4 mM MgCl2, 20 mM imidazole (pH 7.00), 14.5 mM creatine phosphate, and 4.7 mM ATP. The relaxing solution contained CaCl2 to achieve pCa 8.0 (−log [Ca2+]i), where [Ca2+]i is calcium concentration, and the maximal activating solution had pCa 3.8. In addition, a series of activating solutions were made to vary the ionized calcium concentration at 0.1 pCa intervals from pCa 3.8 to pCa 6.0 by changing the concentration of CaCl2. All solutions had enough KCl to achieve an ionic strength of 180 mM, and pH was adjusted to 7.00 with KOH.

Determination of Fiber Diameter

The single fiber was observed through an inverted microscope. The segment length was adjusted to a sarcomere spacing of 2.4 μm in relaxing solution with the use of a calibrated eyepiece. The fiber diameter was determined as the mean of three measurements made along the length of the fiber, and the fiber cross-sectional area was calculated by assuming a circular cross section.

Determination of Peak Active Force and Peak Specific Tension

The output of the force transducer was amplified and sent to a microcomputer via a universal input-output board and to an oscilloscope for immediate examination. Data were collected, analyzed, and stored by customized software (3). To determine peak active force, the baseline passive force output, in relaxing solution (pCa 8), was monitored. The fiber was then transferred into activating solution (pCa 3.8), and peak active force of the fiber was determined. The peak specific tension, defined as the peak active force normalized to fiber cross-sectional area, was expressed in kilonewtons per meter squared.

Determination of pCa-Force Measurements

The pCa-force relationship was determined in a series of activating solutions in which the pCa ranged from 3.8 to 6.0. Submaximal force values were expressed relative to peak active force; that is, P = peak
submaximal force/peak active force at pCa = 3.8. The contractile response of fibers to calcium were determined by performing Hill linear transformations, using the equation log [P/(1 – P)] vs. −log [Ca²⁺]. Hill plot coefficients, n₁ and n₂, were calculated as the slope of the least squares regression line fit to points P₁ > 0.50 and P₂ < 0.50, respectively. The [Ca²⁺] eliciting half-maximal activation (pCa₅₀) was calculated as the mean x-intercept for each Hill plot (20). The Ca²⁺ activation threshold was calculated as the [Ca²⁺], where log [P/(1 – P)] = −2.5, reflecting the minimum amount of calcium necessary to initiate force generation.

Fiber-type Identification and MHC Analysis

After physiological measurements were made, the MHC isof orm composition of each fiber was determined by SDS-PAGE. Each fiber was solubilized in 100 μl of sample buffer containing 1% SDS, 6 mg/ml EDTA, 60 mM Tris-HCl (pH 6.8), 2 mg bromophenol blue/ml, 15% glycerol, and 5% β-mercaptoethanol and then stored at −80°C. In brief, 5 μl of the buffer that contained the solubilized fiber were loaded on gels containing a 3% acrylamide stacking gel and a 5% separating gel. Each muscle fiber was typed by comparison of its MHC mobility to that of lanes containing a standard made from homogenates of rat tibialis anterior muscle that contained all four MHC isoforms (I, IIA, IX, and IIB).

Statistical Analysis

Data are presented as means ± SE. An ANOVA was performed to determine differences by MHC fiber type within each group, and when a significantly different F value was observed, a pairwise Tukey honestly significant difference analysis was performed. MHC type I fibers were analyzed exclusively from the soleus muscle, and MHC type II fibers were analyzed exclusively from the EDL muscle. Fibers from the EDL muscle expressing MHC type IIB and coexpressing both MHC types IIX and IIB (IIX/IIB) from the same group were not significantly different in fiber diameter, peak force, specific tension, activation threshold, or pCa₅₀. Thus these fiber types were pooled together. By doing this, any difference between groups was due to the effects of exercise or diabetes and not to variations in fiber-type distribution between groups. Although other MHC fiber types were present in the muscles, we did not include them in the analysis because the sample size was inadequate and/or the representation of the particular fiber type was not sampled from each muscle.

Univariate general linear model procedure was used to determine main effect for activity (exercise vs. sedentary), main effect for disease (diabetes vs. nondiabetes), and interactions between activity and disease in fibers expressing either MHC type I from the soleus muscle or in fibers expressing MHC type IIB and IIX/IIB from the EDL muscle. When significant interactions were found, Student t-tests were performed for activity and disease. Significance was set at a P < 0.05. All statistical analyses were performed using the statistical software SPSS 11.5.

RESULTS

Blood Glucose, Amount of Insulin Administration, Blood Lactates, and Citrate Synthase Activity

Blood glucose was not significantly different between diabetic rats, but less insulin was required to maintain the same blood glucose levels in diabetic rats that exercised compared with diabetic rats that were sedentary (Table 1). After an acute bout of treadmill running at the end of the 12 wk of endurance exercise training, blood lactate values were twice as high in the sedentary groups as in the exercise groups (5.91 ± 3.48 vs. 2.86 ± 1.05 mmol/l, respectively). Citrate synthase activity was not significantly different between diabetic and nondiabetic rats. Therefore, the data were pooled. Citrate synthase activity was significantly greater in the white gastrocnemius muscle from the exercise groups (10.8 μmol·g⁻¹·min⁻¹) than the sedentary groups (7.8 μmol·g⁻¹·min⁻¹).

MHC Isoform Distribution of the Single Fibers

Fiber-type distribution followed expected values for the percentage of MHC fiber types in rat soleus and EDL muscles (7, 13). The EDL muscle was composed of 99% fibers expressing the different type II MHC isoforms, of which fibers expressing type IIX and IIX/IIB MHC isoforms represented ~75% of the single fibers analyzed for contractile properties. The soleus muscle was composed of 90% of fibers expressing type I MHC isoform, and the other 10% were from fibers expressing type IIA MHC isoform.

Fiber Diameter, Peak Force, and Specific Tension

Data from muscle fibers expressing type IIB and coexpressing type IIX-IIB MHC isoforms from the EDL muscle did not show fiber-type-specific contractile properties; thus the data were pooled. Fibers expressing type I MHC from soleus muscles were analyzed separately. Figure 1, Table 2, and Table 3 summarize single-fiber contractile characteristics for type II fibers from the EDL muscles and type I fibers from the soleus muscles.

Diameter. There was an interaction between the disease state of the rat and activity level for type II fiber diameter (Fig. 1A). The endurance exercise program induced single-fiber atrophy in the EDL muscles of nondiabetic rats, whereas no atrophy was observed in those muscles from diabetic rats. In addition, diabetes resulted in significant cellular atrophy when rats are sedentary, whereas no atrophy was observed in exercising rats. In the type I fibers, diameter was not influenced by activity or disease (Table 2).

Force. Consistent with fiber atrophy, we observed an 11% lower peak force in type II fibers from diabetic rats (Fig. 1B). In soleus fibers expressing type I MHC, the force-generating capacity was not affected by exercise or diabetes (Table 2).

Specific tension. Specific tension of type II fibers was 13% greater in the exercise groups than type II fibers from sedentary groups (Fig. 1C). The specific tension of the type I fibers was 20% lower in the exercise groups than in the sedentary groups (Table 2).

Activation Threshold and pCa₅₀

In type II fibers, activation threshold was unaffected by diabetes or exercise. pCa₅₀ was significantly greater with endurance training in both diabetic and nondiabetic rats relative to their sedentary counterparts (Table 3).
The present study confirms that the decrease in force observed in single muscle fibers from insulin-treated STZ-induced diabetic rats is due to cellular atrophy and not to changes in the quality of the contractile proteins because specific tension did not change with diabetes (37). More importantly, the results demonstrate that endurance exercise training does not induce further skeletal muscle fiber atrophy in the rats with diabetes. It is important to note that the effects of a total lack of insulin were not evaluated in this study because the rats were insulin treated. Thus the interpretation of the results must take this into consideration. In addition, blood glucose values were not significantly different between the two diabetic conditions, which argues in favor of the conclusion that the dose of insulin given to groups of diabetic rats had a similar effect in controlling glucose metabolism and thus on the severity of diabetes on long-term complications. Therefore, the main difference between both groups was the effect of endurance exercise training.

**Metabolic Adaptations to Endurance Exercise Training**

Endurance-exercised athletes have lower blood lactate values than sedentary individuals after a bout of aerobic exercise at the same intensity (29). A decrease in lactate production due to an increase in aerobic capacity and an increase in blood lactate clearance are the two most important factors that explain the decrease in blood lactate values in endurance-trained athletes (45). The lower blood lactate values in the exercise-trained rats compared with the sedentary rats in this study and the 38% greater citrate synthase activity in the white gastrocnemius muscle from the endurance-exercised rats are indicative of cellular exercise-induced adaptations with this training program.

**Type I Fibers vs. Type II Fibers**

Diabetes preferentially affected diameter and peak force of fibers from the EDL muscle (type II fibers), because fibers expressing type I MHC isoform from the soleus did not atrophy. The mechanism that may explain the difference in fiber-size adaptations between fibers from the soleus and EDL muscles from diabetic rats is that, in the weight-bearing animal, fibers from the soleus muscle are constantly being used (2, 25). This frequent activation of muscle fibers might prevent fiber atrophy caused by the increased catabolism that is characteristic of diabetes. In addition, the greater blood perfusion to the skeletal muscles from the endurance-exercised rats are indicative of cellular exercise-induced adaptations with this training program.

**Table 2. Single-fiber contractile characteristics of type I fibers from the soleus muscles**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Diameter (µm)</th>
<th>Peak Force (mg)</th>
<th>Specific Tension (kN/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic (30–31)</td>
<td>80±2</td>
<td>76±3</td>
<td>154±7</td>
</tr>
<tr>
<td>Nondiabetic (16–17)</td>
<td>84±3</td>
<td>66±4</td>
<td>123±12*</td>
</tr>
<tr>
<td>Diabetic (25)</td>
<td>79±3</td>
<td>69±5</td>
<td>135±8</td>
</tr>
<tr>
<td>Diabetic (13)</td>
<td>85±3</td>
<td>65±7</td>
<td>111±6*</td>
</tr>
</tbody>
</table>

Values are means ± SE of individual type I fibers (fibers expressing type I myosin heavy chain isoform). No. of individual fibers examined in parentheses. *Main effect for activity, *P* = 0.006.
In the nondiabetic exercise rats, atrophy was observed in fibers expressing type IIB and IIX/IIB MHC isoforms from the EDL muscle, but not in fibers expressing type I MHC isoforms from the soleus muscle. This is probably due to the different mechanical loads experienced by the two muscles during a walking cycle. That is, the soleus muscle performs an isometric contraction while the EDL muscle performs an unloaded contraction during walking (27). In support of this explanation, Fitts et al. (39) reported no differences in fiber diameter between sedentary and endurance exercise-trained rats in the soleus (type I, IIA) or gastrocnemius (type IIA, IIB) muscles. Similarly, Holloszy et al. (9) reported that muscle mass and cross-sectional area were greater in the soleus muscle after 5 and 23 mo of voluntary wheel running compared with age-matched sedentary rats. The results from these studies and our study show that the effects of exercise on type IIB and IIX/IIB MHC fibers are not only dependent on the characteristics of the exercise (endurance vs. resistance) but are also dependent on the particular mechanical function of the muscle. Thus information obtained from type II fibers from the EDL muscle must be extrapolated with caution to fibers expressing type II MHC isoforms from other muscles performing different mechanical functions.

**Atrophy and Force-generating Capacity**

Specific tension was greater in type II fibers from the endurance exercise-trained rats relative to the sedentary rats. From the data obtained in this study, we can only speculate on the mechanisms that may explain this greater specific tension. Although an increase in specific tension can result from an increase in the fraction of cross bridges in the strong-binding structural state or an increase in the force generation of each individual cross bridge, these two mechanisms are not likely because a previous study demonstrated that exercise does not modify the structure of myosin (30).

In general, the breakdown of noncontractile proteins can result in fiber atrophy without a significant decrease in force production because the myosin-actin interactions are not perturbed (36). In the nondiabetic exercise group, we observed a significant decrease in fiber diameter, but peak force was unaffected. This decrease in fiber cross-sectional area and maintenance of peak force may be the cause for the increase in specific tension (Fig. 1). The decrease in fiber cross-sectional area is part of the adaptation process of muscle fibers to endurance exercise training, which includes an increase in capillarity, increase in mitochondrial density, increase in aerobic enzyme activity and lipid oxidation, and modification of type II fibers to resemble the characteristics of type I muscle fibers (34). Thus the decrease in fiber size with endurance training can be viewed as a benefit because it improves the delivery and utilization of oxygen.

Cellular atrophy in the diabetic state (sedentary rats) is related to a combination of diabetic neuropathy, an increase in the catabolic state, and the lack of the effects of insulin to promote protein synthesis and inhibit protein breakdown (15, 22, 32, 40, 41). During a state of absolute or relative insulin deficiency, there is an increase in the rate of protein degradation of both structural and contractile proteins due to activation of the ubiquitin-proteasome pathway and also a decrease in the rate of protein synthesis (17, 40). Those alterations in protein turnover promote cellular atrophy. In contrast, single-fiber atrophy is partially reversed by the administration of insulin, most likely due to sustaining protein synthesis (17). Thus the decrease in fiber size with the diabetic state may be viewed as negative in the sedentary animal.

Before the present study, it was unclear whether endurance training would magnify fiber atrophy in the diabetic state. It is possible to speculate that enhanced cellular atrophy would occur when combining endurance training and diabetes because both conditions promote atrophy individually. However, the results of the present study suggest that skeletal muscle fiber atrophy is not enhanced when an insulin-treated diabetic animal performs an aerobic training protocol. The underlying cellular mechanisms most likely involve sustaining protein synthesis rates in the working muscles and minimal changes in degradation rates.

**Activation Threshold and pCa50**

The pCa-force relationship is used to investigate calcium sensitivity. Two experimental parameters evaluated from this relationship are activation threshold and pCa50. In fibers expressing type IIX and IIX/IIB MHC isoforms, diabetes does not change activation threshold, but pCa50 is significantly higher compared with fibers from nondiabetic rats (37). An increase in pCa50 may be due to changes in troponin C, regulatory light chain phosphorylation, fiber sarcomere length, pH, ionic strength, Pi, or temperature during the contraction process (31). Fiber sarcomere length, pH, ionic strength, Pi, and temperature were all controlled in our experimental protocols. Therefore, changes in either troponin C isoform or in the regulatory light chain phosphorylation status may be implicated for the increase in pCa50 observed in fibers expressing type IIX and IIX/IIB MHC isoforms from diabetic rats. It is possible that changes in the expression of troponin C from a fast to a slow isoform may have been induced by endurance exercise training (26). Endurance training in healthy rats results in changes in both activation threshold and pCa50 (21). Specifically, type II skeletal muscle fibers show an increase in

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**Table 3. Calcium sensitivity characteristics**

<table>
<thead>
<tr>
<th>Type II Fibers (EDL)</th>
<th></th>
<th>Type I Fibers (Soleus)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation threshold, pCa</td>
<td></td>
<td>Activation threshold, pCa</td>
<td></td>
</tr>
<tr>
<td>Nondiabetic sedentary</td>
<td>6.14±0.03</td>
<td>5.54±0.02</td>
<td>6.70±0.08</td>
</tr>
<tr>
<td>Nondiabetic exercise</td>
<td>6.26±0.03</td>
<td>5.69±0.04*</td>
<td>6.54±0.05</td>
</tr>
<tr>
<td>Diabetic sedentary</td>
<td>6.20±0.03</td>
<td>5.58±0.02</td>
<td>6.52±0.09</td>
</tr>
<tr>
<td>Diabetic exercise</td>
<td>6.21±0.02</td>
<td>5.68±0.03*</td>
<td>6.55±0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE of individual type II fibers (fibers expressing type IIB, coexpressing type IIB/IIX myosin heavy chain isoforms, n = 218) from the extensor digitorum longus (EDL) muscles and type I fibers (n = 86) from the soleus muscles. *Main effect for activity, P < 0.035.
sensitivity to calcium after an endurance training program (21, 34). These endurance-training-induced changes in the type II fibers resemble the contractile properties of type I fibers. In the present study, the combination of diabetes and endurance training had an influence on pCa50, which is also consistent with endurance training-induced changes in contractile properties of type II fibers (21, 34). Although it is difficult to conclude whether these skeletal muscle fiber changes are beneficial, the findings do indicate that single skeletal muscle fibers maintain their ability to adapt to an endurance training protocol when the animal is in a diabetic state receiving insulin.

Clinical Implications

Endurance exercise training is an essential component for the treatment of diabetes mellitus because it helps regulate blood glucose and decreases the risk of long-term cardiovascular complications (38). However, because decreased muscle strength has been observed in patients with long-term diabetes, endurance exercise training might not be the most appropriate exercise option for this group of individuals. Instead, resistance exercise, which is capable of inducing significant muscle hypertrophy and increasing strength, even in moderately diabetic individuals with low serum levels of insulin, might be a more appropriate exercise training option for patients with long-term diabetes and associated muscle weakness (4, 6, 16, 17). In addition, resistance exercise can also improve HbA1c, a marker of long-term glucose management, decrease insulin resistance, and lower risk factors for cardiovascular disease (38). Resistance exercise has been proven to increase muscle strength in elderly patients, which can be explained by an increase in fiber diameter and greater peak force production by individual muscle fibers (19, 23). This increase in force was associated with improvements in performing activities of daily living (19). Therefore, a future study should be designed to determine whether resistance exercise can increase muscle strength in diabetic patients with low muscle strength.

In summary, muscle weakness is caused by atrophy of individual muscle cells in STZ-induced insulin-treated diabetic rats and not by changes in the properties of the contractile proteins, since specific tension does not decrease. Endurance exercise does not appear to magnify skeletal muscle atrophy in the diabetic rats.

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GRANTS

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