

Original Article

Abnormalities in the fiber composition and contractility in diabetic skeletal muscles

Lina Huang, Lianhua Chen, Yimin Qiu, Shitong Li

Department of Anesthesiology, The Affiliated First People's Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 201620, China

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Abstract: The study aimed to investigate the changes induced by diabetes in diaphragm (DIA), extensor digitorum-longus (EDL), and soleus (SOL) contractility and histochemistry throughout a broad time span. The contractility and ATPase staining of DIA, EDL, and SOL from the streptozotocin-induced rats and age-matched control rats were observed over a period ranging from 4 weeks to 16 weeks after streptozotocin treatment. Only the muscle weight-to-body weight ratio of EDL displayed a reduction. Twitch tension force and tetanic tension decreased, while the time to peak tension and half-relaxation time were prolonged in diabetic rats. The fatigue index of DIA demonstrated an increase from 4 weeks to 8 weeks and a decline from 12 weeks to 16 weeks after the induction of diabetes. Regarding fatigue indexes, EDL and SOL demonstrated an incremental trend during the time frame. For DIA and SOL in the diabetic rats, the proportion of type I fibers grew higher, while EDL revealed a similar composition as the control rats. Both the fiber areas of type I and type II fibers decreased in the diabetic rats. DIA, EDL, and SOL appeared to suffer from muscle atrophy. The contractile forces and speed-related properties were impaired in the diabetic rats. The increased fatigability was demonstrated in DIA at a later stage, whereas resistance to fatigue in DIA at an early stage of diabetes was noted. The endurance to fatigue decreased in EDL and SOL as well. The study indicated that the differential alterations of contractile properties were associated with streptozotocin-induced fiber transformation.

Keywords: Streptozotocin, diaphragm, extensor digitorum longus, soleus, diabetes

Introduction

Diabetes Mellitus (DM) is a metabolic disorder characterized by abnormally high blood glucose levels due to decreased secretion or effectiveness in the function of insulin. Diabetes, if poorly controlled, leads to skeletal muscle atrophy, decreasing the quality of life [1-3]. Recently, diabetic myopathy has received much attention [4-6]. The pathophysiology of diabetic myopathy includes reduced physical capacity, as well as loss of muscle mass and contractile function [4, 7, 8].

Skeletal muscles are composed of heterogeneous types of fibers based on the expression of different myosin heavy chain (MyHC) isoforms [9, 10]. Skeletal muscle fibers are classified into three types: slow-twitch oxidative (type I) containing only the slow MyHC isoform, whereas fast-twitch oxidative-glycolytic (type

IIA) and fast-twitch glycolytic (FG, type IIB) skeletal muscle fibers containing the fast MyHC isoforms [6]. Extensor digitorum longus (EDL) muscle and soleus (SOL) muscle are typical fast and slow twitch muscles, composed of predominately fast and slow twitch fibers, respectively. Regarding diaphragm (DIA), it is a kind of respiratory muscle, composed of mixed twitch fibers, and is considered a limiting factor for overall muscle performance [11].

Muscle contractile properties depend on the alterations of muscle types [7, 8]. The typing of muscle fibers is based on oxidative and glycolytic capacities, twitch characteristics, and ATPase activity [12]. Besides the effects of different muscle fiber types, muscle contractile force is directly related to intracellular calcium ion (Ca^{2+}) levels. The more Ca^{2+} is released from the sarcoplasmic reticulum (SR), the higher the contractile force becomes due to the saturation

of contractile proteins by Ca^{2+} [11]. The SR density in type II fibers is twice than that of type I fibers, which may be one reason for different contractile properties [13]. Moreover, it has been shown that in fast twitch fibers the SR had a larger capacity and the endogenous Ca^{2+} content was a relatively lower percentage of maximum compared with slow twitch fibers [14]. Diabetic human and animals have been reported to show an increase in type I fibers and a reduction in type IIA and IIB fibers in several muscles [6, 7, 15]. Due to these, the contractile properties in diabetes may be muscle specific [7, 8, 16, 17].

It has been demonstrated that the progress in muscle functions and fiber alteration occurs in a time-dependent manner [18]. The duration of diabetes may markedly influence the performance of the complication. However, few studies have directly assessed the progressive alterations of skeletal muscles in DM under the same conditions.

Streptozotocin (STZ) is a diabetogenic agent that had been widely used in animal models to study insulin-dependent DM and had demonstrated a wide variety of physiological effects on peripheral nerves and muscles [19]. The STZ-induced diabetes is a well-known model of type 1 DM, which has a distinct etiology from type 2 DM. Aside from the distinctions, the alterations in muscle structures associated with reductions in muscle functions are quite similar [20]. So the findings of the current study are of great clinical relevance.

The aim of the current study was to investigate the changes induced by diabetes in DIA, EDL, and SOL contractility and histochemistry throughout a broad time span. This study observed STZ-induced diabetic rats and age-matched control rats over a period ranging from 4 weeks to 16 weeks after STZ treatment.

Materials and methods

Animals

This study was approved by the Animal Care and Use Committee of the Shanghai Jiaotong University School of Medicine and was performed in compliance with the World Health Organization International Guiding Principles

for Animal Research. A total of 48 male Sprague-Dawley rats (Experimental Animal Center of the School of Medicine, Shanghai Jiaotong University, Shanghai, China), weighing 220-260 g, were housed in groups of three. They were fasted but allowed to have free access to water and food prior to the experiments.

Induction of diabetes

Rats were randomly divided into two groups. One group ($n=24$) was the diabetic group, which was induced by a single intraperitoneal injection of STZ (60 mg/kg, Sigma, St Louis, MO, USA) in 20 mm sodium citrate buffer (pH 4-5) after 12 hours fasting. Another group ($n=24$) was the normal group, which comprised normal animals matched in age and was given the same volumes of citric acid buffer solution only. Two days after injection, blood sugar was measured twice in series by cutting tails. Successful models were determined if the blood sugar values were above 16.7 mmol/L. The rats with blood sugar tested below 16.7 mmol/L were rejected from the study. After modeling successfully, the diabetic rats and the age-matched control rats were randomly divided into four subgroups: diabetic 4 weeks group (4wDM), diabetic 8 weeks group (8wDM), diabetic 12 weeks group (12wDM), and diabetic 16 weeks (16wDM) group and control 4 weeks group (4wC), control 8 weeks group (8wC), control 12 weeks group (12wC), and control 16 weeks group (16wC) ($n=6$ each group), respectively. The rats were sacrificed at 4 weeks, 8 weeks, 12 weeks, and 16 weeks after STZ administration.

Muscle preparations

Rats were killed with 60 mg/kg pentobarbital intraperitoneally. Strips of DIA, EDL, and SOL were established for direct electrical stimulation as described previously [8, 21, 22]. The body temperature was maintained at 37°C using a heating blanket and radiant heat.

Strips of DIA with a central tendon and rib cage intact (5 mm wide), which were cut parallel to the muscle fibers, were rapidly removed from each of the rats for morphological analysis. Either the intact EDL or the SOL was exposed in one leg. After measurements were completed for one muscle, the other was then exposed in

the other leg. The SOL was exposed by sectioning the tendons connecting the plantaris and gastrocnemius muscles to the heel, and reflecting the muscles back. A silk thread was attached to the distal tendon of the SOL, and the tendon was sectioned. The muscle was then carefully freed of surrounding tissues, ensuring the blood supply remained intact. The EDL was prepared in the similar manner after first exposing the muscle by reflection of the anterior tibialis muscle. The silk sutures were tied to the proximal and distal tendons of the EDL and/or SOL muscles, and the muscles were removed, tendon to tendon. The EDL, SOL, and DIA of the other side were dissected simultaneously for ATPase staining.

The isolated muscle preparations were dipped immediately into plexiglass chambers filled with Krebs solution, maintained at 37°C and bubbled with 95% oxygen/5% CO₂. The composition of the Krebs solution was as follows: 137 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 1 mM KH₂PO₄, 12 mM NaHCO₃, and 6.5 mM glucose, with a pH 7.40±0.05 during bubbling.

Muscle contractile properties

The electrical stimulation-evoked twitch tension was recorded with MPA Multiple Channel Biological Signal Analysis System (provided by the Department of Anesthesiology, Shanghai First People's Hospital, Shanghai Jiaotong University). Each isolated strip was mounted vertically in a tissue chamber, inferiorly positioned. One side of the isolated DIA preparation was fixed at the rib cage; meanwhile, the other side was suspended at the central tendon from a force displacement transducer using a 3-0 silk suture (ALC-M System for Isolated Tissue-Organ Research, Acquisition and Analysis System for Life Science Research, Shanghai Alcott Biotech, Shanghai, China; 40 mL in volume). As for the EDL and SOL preparations, they were aligned vertically with a distal tendon attached to the force displacement transducer and a proximal tendon fixed to the stainless steel fixed-post. The chamber was filled with Krebs solution as mentioned earlier. The muscle strip was placed between two large silver electrodes and pretreated with a sufficient concentration (12 µM) of d-tubocurarine to completely eliminate neuromuscular transmission.

Isometric tension was elicited by direct supra-maximal constant-voltage stimulation at 0.1 Hz for 0.2 ms, using a stimulator and a constant-voltage unit. The twitch tension was recorded via the force transducer on a recorder (ALC-MPA 2000 m, Acquisition and Analysis System for Life Science Research, Shanghai Alcott Biotech, Shanghai, China). The stimulator was activated by a personal computer. Twitch stimuli were used to determine the optimal length (LO) at which skeletal muscle may generate the greatest force. A number of measurements were performed as described in the subsequent sections.

Twitch tension

Three twitches were recorded at LO to obtain maximal twitch force (P_t), timetopeak tension (TPT), and half-relaxation time (HRT). The average values were calculated for analysis.

Tetanic tension

Two supramaximal stimuli with a frequency of 120 Hz for 250 ms at an optimal length were generated to obtain the maximal tetanic force (P_o) and twitch tension-to-tetanic force ratio (P_t/P_o).

Fatigue

Muscle strips were fatigued by means of 330 ms stimulations repeated at 40 Hz and applied every second for 5 minutes. Fatigue index (FI) is defined as the ratio of tension after 5-minute fatigue protocol to maximal isometric tension [22].

Following the experiment, the muscles were weighed and the cross-sectional area (CSA) was estimated based on the formulas as follows: for DIA, CSA (cm²)=muscle weight (g)/[LO (cm)×1.056 (g/cm³)], assuming muscle density as 1.056 g/cm³ [21]; for EDL and SOL, CSA (cm²)=muscle weight (g)/[LO (cm)×1 (g/cm³)], assuming muscle density as 1 g/cm³ [22]. Absolute force, normalized per CSA (in N/cm²) was determined at the end of the equilibration period.

Muscle histochemical study

The strips for ATPase staining were embedded separately in plastic holders and rapidly frozen in isopentane cooled in liquid nitrogen (-160°C),

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Table 1. Body and muscle weights from the diabetic rats and age-matched controls

Variables	4 weeks		8 weeks		12 weeks		16 weeks	
	Control	Diabetic	Control	Diabetic	Control	Diabetic	Control	Diabetic
Body weight (g)	378.9±72.4	260.0±93.9*	431.7±64.8	224.7±39.4*	490.6±49.6	216.7±45.9*	496.4±72.4	218.3±29.1*
DIA weight (g)	0.074±0.013	0.052±1.342*	0.081±0.015	0.043±0.014*	0.083±0.028	0.036±0.010*	0.084±0.013	0.033±0.012*
DIA M/B ratio (×10 ⁻⁴)	1.95±0.42	2.00±0.43	1.88±0.36	1.91±0.41	1.69±0.33	1.66±0.46	1.69±0.37	1.51±0.41
EDL weight (g)	0.157±0.011	0.098±0.015*	0.179±0.033	0.081±0.014*	0.210±0.088	0.074±0.150*	0.214±0.013	0.067±0.007*
EDL M/B ratio (×10 ⁻⁴)	4.14±0.32	3.77±0.57*	4.15±0.54	3.60±0.43*	4.28±0.31	3.41±0.34*	4.31±0.33	3.06±0.24*
SOL weight (g)	0.145±0.041	0.107±0.134*	0.171±0.032	0.097±0.015*	0.196±0.018	0.089±0.006*	0.197±0.020	0.089±0.010*
SOL M/B ratio (×10 ⁻⁴)	3.82±0.45	4.11±0.52*	3.96±0.39	4.31±0.37*	4.00±0.29	4.11±0.43	3.97±0.41	4.07±0.40

Values are expressed as mean ± SD. Statistical analysis was performed by one-way ANOVA with post-hoc Bonferroni testing. **P*<0.05 versus age-matched controls. ANOVA, Analysis of variance; DIA, diaphragm; EDL, extensor digitorum longus; SOL, soleus; M/B ratio, muscle weight to body weight ratio.

Table 2. Contractile properties of diaphragm from the diabetic rats and age-matched controls

Variables	4 weeks		8 weeks		12 weeks		16 weeks	
	Control	Diabetic	Control	Diabetic	Control	Diabetic	Control	Diabetic
P _i (N/cm ²)	3.50 (0.90)	3.33 (1.11)	3.41 (1.04)	3.10 (0.87)*	3.53 (0.92)	3.02 (0.89)*	3.49 (0.95)	2.87 (0.99)*
TPT (ms)	20.3 (1.9)	24.5 (2.8)*	21.2 (2.3)	25.7 (2.5)*	20.7 (1.9)	26.9 (2.3)*	21.0 (2.8)	28.8 (2.6)*
HRT (ms)	21.9 (1.8)	25.3 (2.2)*	22.0 (2.1)	26.9 (3.0)*	21.0 (2.7)	27.8 (2.1)*	20.9 (2.5)	29.7 (2.5)*
P _o (N/cm ²)	17.5 (2.3)	15.1 (2.8)*	16.2 (3.0)	11.7 (2.4)*	14.1 (2.7)	11.2 (1.9)*	15.2 (3.2)	10.3 (2.3)*
P _i /P _o	0.20 (0.06)	0.22 (0.05)	0.21 (0.07)	0.23 (0.09)	0.25 (0.08)	0.27 (0.12)	0.23 (0.11)	0.28 (0.09)*
FI (%)	35.2 (8.6)	50.2 (7.2)*	34.6 (8.3)	56.9 (9.2)*	38.2 (8.1)	23.5 (5.7)*	37.3 (6.8)	14.8 (8.3)*

Data are presented as mean (SD). Statistical analysis was performed by one-way ANOVA with post hoc Bonferroni testing. **P*<0.05 versus age-matched controls. ANOVA, Analysis of variance; P_i, twitch tension; TPT, time to peak tension; HRT, half-relaxation time; P_o, tetanic tension; P_i/P_o, twitch/tetanic ratio. FI, fatigue index, the ratio of tension after 5-minute fatigue protocol to maximal isometric tension.

followed by further freezing in liquid nitrogen. Muscle blocks were serially sliced at 15 µm on a cryostat (Leica CM1850, Nussloch, Germany). The modified ATPase staining method was applied in addition to hematoxylin and eosin staining.

Myofibrillar ATPase staining was done following alkaline pH 10.4 and acid pH 4.5 using a modification of the procedure described by Brooke and Kaiser [23]. Fibers were classified into type I (slow-twitch muscle fiber with weak mATPase activity) and type II (fast-twitch muscle fiber with strong mATPase activity) [12]. The image analysis software Image-Pro Plus, version 5.1 (Media Cybernetics, MD, USA) was used to evaluate the prepared samples. Then, approximately 400 fibers per muscle were measured for muscle fiber type distribution and CSA (µm²) of each fiber type. The distribution of muscle fibers of a given fiber type (type I or II) was expressed as a percentage of all the fibers in the section.

Statistical analysis

All data were analyzed by SPSS version 13.0 software (IBM, NY, USA). Data were expressed as mean ± standard deviation (SD). One-way

analysis of variance was used to test the significance of differences among all groups. The paired *t* test was used for comparison between control group and diabetic group in the same time groups. *P*<0.05 was considered to indicate a statistically significant difference.

Results

Blood glucose levels

At the time of sacrifice, the level of blood glucose was measured by glucose-strip reader and found to be 25.01±4.05 versus 4.90±0.53 mmol/L for the 4Wdm group and the age-matched control animals, respectively (*n*=6 for each experimental group, *P*<0.01), not increasing significantly with diabetes progression, 26.30±5.01 versus 5.31±1.51 mmol/L in 8wDM rats and 8wC, 25.60±4.91 versus 5.53±1.72 mmol/L in 12wDM rats and 12wC, 26.11±4.59 versus 5.44±1.63 mmol/L in 16wDM rats and 16wC, respectively (*n*=6 for each group, *P*<0.01).

Body and muscle weight

Body weights of the control rats showed a steady increase from 378.9±72.4 g (4wC) to

Table 3. Contractile properties of extensor digitorum longus from the diabetic rats and age-matched controls

Variables	4 weeks		8 weeks		12 weeks		16 weeks	
	Control	Diabetic	Control	Diabetic	Control	Diabetic	Control	Diabetic
P_t (N/cm ²)	4.04 (0.84)	3.66 (0.90)*	4.11 (0.93)	3.70 (0.89)*	4.21 (0.79)	3.68 (0.97)*	4.17 (0.91)	3.45 (0.84)*
TPT (ms)	13.4 (0.7)	14.2 (1.1)	13.2 (1.3)	13.9 (0.9)	13.6 (1.1)	14.6 (1.2)*	13.7 (0.8)	15.0 (1.8)*
HRT (ms)	12.9 (0.5)	13.4 (1.4)	13.1 (1.2)	13.7 (1.0)	13.0 (1.3)	14.0 (1.2)*	13.1 (1.1)	14.8 (1.3)*
P_0 (N/cm ²)	50.1 (8.4)	38.6 (7.3)*	58.7 (12.4)	36.4 (16.3)*	46.8 (7.8)	30.6 (15.3)*	52.1 (12.5)	28.9 (8.9)*
P_t/P_0	0.08 (0.02)	0.09 (0.03)	0.07 (0.03)	0.10 (0.04)*	0.09 (0.05)	0.12 (0.04)*	0.08 (0.03)	0.12 (0.01)*
FI (%)	56.3 (8.2)	50 (9.3)*	55.5 (10.2)	45.8 (7.6)*	56.1 (11.1)	40.1 (8.4)*	53.7 (9.6)	34.6 (8.7)*

Data are presented as mean (SD). Statistical analysis was performed by one-way ANOVA with post hoc Bonferroni testing. * $P < 0.05$ versus age-matched controls. ANOVA, Analysis of variance; P_t , twitch tension; TPT, time to peak tension; HRT, half-relaxation time; P_0 , tetanic tension; P_t/P_0 , twitch/tetanic ratio. FI, fatigue index, the ratio of tension after 5-minute fatigue protocol to maximal isometric tension.

Table 4. Contractile properties of soleus from diabetic rats and age-matched controls

Variables	4 weeks		8 weeks		12 weeks		16 weeks	
	Control	Diabetic	Control	Diabetic	Control	Diabetic	Control	Diabetic
P_t (N/cm ²)	5.71 (0.8)	5.29 (0.7)*	5.80 (0.8)	5.31 (0.8)*	5.67 (1.0)	5.10 (0.9)*	5.62 (1.1)	5.02 (1.2)*
TPT (ms)	30.8 (2.8)	34.7 (4.5)*	32.3 (3.6)	36.8 (4.2)*	31.3 (3.9)	38.7 (7.0)*	30.9 (7.3)	42.1 (10.9)*
HRT (ms)	29.5 (4.3)	35.7 (4.7)*	30.2 (5.9)	38.6 (7.3)*	33.1 (7.2)	40.6 (8.3)*	32.6 (6.7)	44.6 (7.2)*
P_0 (N/cm ²)	31.7 (12.2)	25.2 (9.1)*	36.2 (10.5)	24.1 (13.1)*	31.5 (9.5)	21.5 (10.2)*	33.1 (8.2)	19.3 (7.9)*
P_t/P_0	0.18 (0.04)	0.21 (0.05)*	0.16 (0.03)	0.22 (0.04)*	0.18 (0.06)	0.24 (0.04)*	0.17 (0.04)	0.26 (0.05)*
FI (%)	82.3 (12.1)	70.1 (15.2)*	80.4 (9.7)	60.3 (10.6)*	78.8 (13.4)	45.8 (7.9)*	76.2 (12.6)	27.1 (13.1)*

Data are presented as mean (SD). Statistical analysis was performed by one-way ANOVA with post hoc Bonferroni testing. * $P < 0.05$ versus age-matched controls. P_t , twitch tension; TPT, time to peak tension; HRT, half-relaxation time; P_0 , tetanic tension; P_t/P_0 , twitch/tetanic ratio. FI, fatigue index, the ratio of tension after 5-minute fatigue protocol to maximal isometric tension.

496.4±72.4 g (16wC), whereas the diabetic rats demonstrated a decline from 260.0±93.9 g (4wDM) to 218.3±29.1 g (16wDM). A significant difference was observed in body weights in the diabetic group compared with that in the age-matched group ($P < 0.05$, **Table 1**).

Muscle weights of DIA, EDL, and SOL for the diabetic rats reduced compared with those for the control rats. While expressed relative to body weight, only the EDL muscle weight-to-body weight ratio (M/B ratio) displayed a reduction ($P < 0.05$, **Table 1**). For SOL, the M/B ratio showed a significant increase compared with the age-matched control rats ($P < 0.05$, **Table 1**). For DIA, the M/B ratio was similar in the diabetic and control animal rats ($P > 0.05$, **Table 1**).

Contractile properties

The results of contractile performances in DIA are shown in **Table 2**. P_t and P_0 expressed per CSA were lower in most of the diabetic rats than in the age-matched control rats. However, P_t was not affected in diabetic rats at 4 weeks after induction. P_t/P_0 showed a higher ratio in

the 16wDM group. TPT and HRT significantly prolonged in DIA of the diabetic rats from 4 weeks to 16 weeks post-treatment of STZ. Additionally, TPT and HRT progressively slowed over the course of the investigation (TPT, 20% by 4 weeks to 37% by 16 weeks; HRT, 16%-30%, respectively). Interestingly, the fatigue index (FI) of DIA demonstrated an increase from 4 weeks to 8 weeks (15.0%-22.3%) and a decline from 12 weeks to 16 weeks (14.7%-22.5%) after induction of diabetes.

The results of contractile measurements in the EDL are shown in **Table 3**. It seemed that P_t and P_0 were significantly reduced and P_t/P_0 was higher in diabetes groups. The increase in TPT and HRT induced by diabetes was recorded in the 12wDM group. Regarding FI in EDL, it demonstrated an incremental trend from 6.3%, 9.7%, and 16.0% to 19.1% during the time frame.

Data of the contractile properties from SOL are summarized in **Table 4**. P_t and P_0 corrected by CSA decreased in the diabetic groups. P_t/P_0 was gradually higher accompanying the prog-

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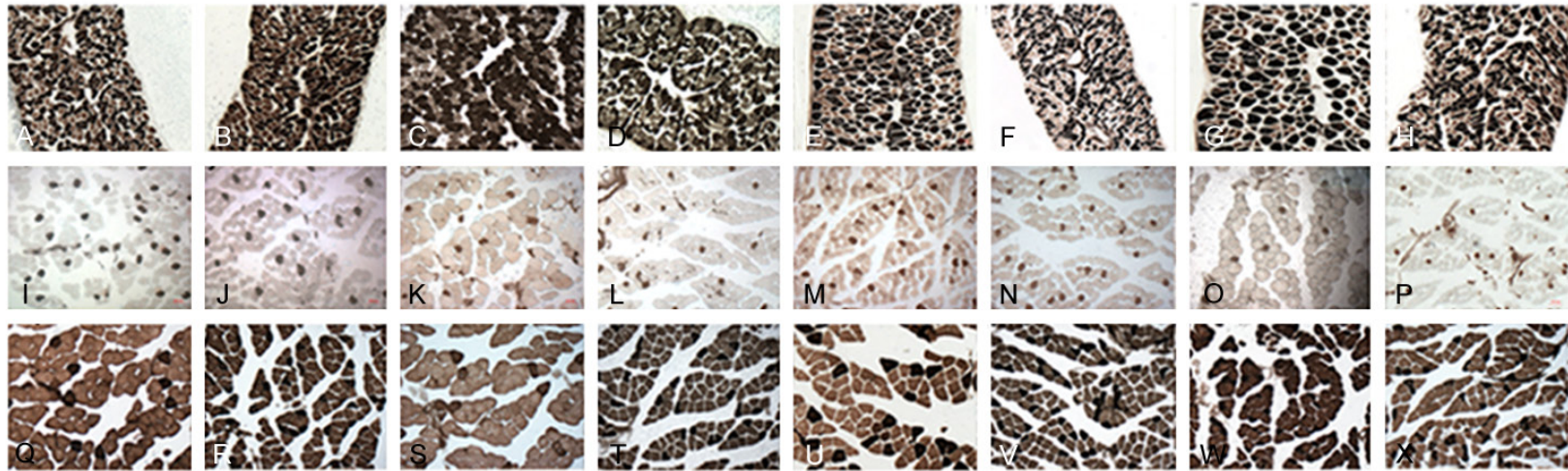


Figure 1. ATPase staining of diaphragm (A-H) and soleus (Q-X) at pH 10.4 and extensor digitorumlongus (I-P) at pH 4.4 from control 4 weeks group (A, I, Q), diabetic 4 weeks group (B, J, R), control 8 weeks group (C, K, S), diabetic 8 weeks group (D, L, T), control 12 weeks group (E, M, U), diabetic 12 weeks group (F, N, V), control 16 weeks group (G, O, W), and diabetic 16 weeks group (H, P, X). The light-colored fibers are type II fibers, while the deep-colored fibers are type I fibers (pH 4.4); the light-colored fibers are type I fibers, while the deep-colored fibers are type II fibers (pH 10.4). (Magnification×100).

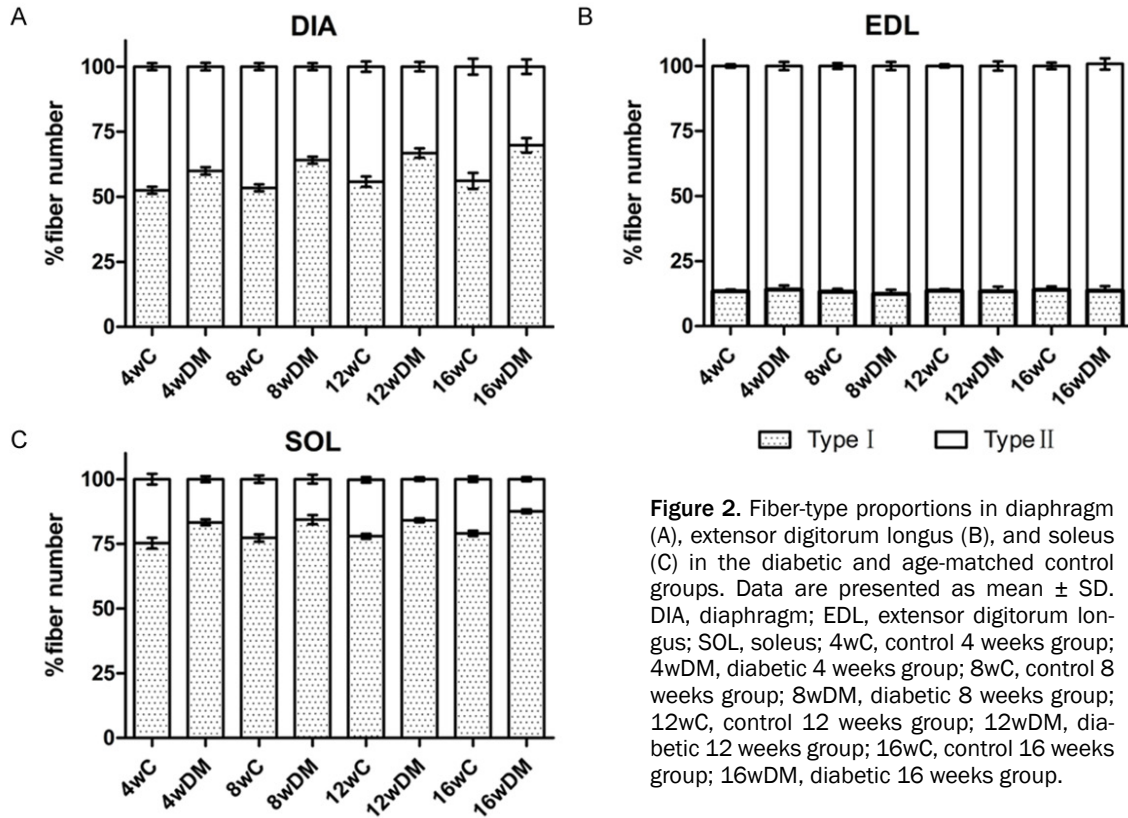


Figure 2. Fiber-type proportions in diaphragm (A), extensor digitorum longus (B), and soleus (C) in the diabetic and age-matched control groups. Data are presented as mean \pm SD. DIA, diaphragm; EDL, extensor digitorum longus; SOL, soleus; 4wC, control 4 weeks group; 4wDM, diabetic 4 weeks group; 8wC, control 8 weeks group; 8wDM, diabetic 8 weeks group; 12wC, control 12 weeks group; 12wDM, diabetic 12 weeks group; 16wC, control 16 weeks group; 16wDM, diabetic 16 weeks group.

ress of diabetes. TPT and HRT were longer in the diabetic rat muscles than in the control rat muscles with the progressive character over the duration of diabetes. The SOL endurance was more pronounced than the EDL endurance in the control rats; however, similar to that in EDL, the decreased endurance induced by diabetes was evidently shown as FI, 12.2% at 4 weeks, 20.1%, 33%, and 49.1% at 8 weeks, 12 weeks, and 16 weeks from experimental models established.

Histochemical study

The morphological analysis of ATPase staining of DIA, EDL, and SOL showed the trend of switching from type II to type I fibers accompanied by a remarkable decrease in fiber areas (Figures 1-3).

The proportion of type I fibers grew steadily higher in the DIA of the diabetic rats than of the age-matched control rats (59.9 ± 2.5 versus $52.5 \pm 2.3\%$ by 4 weeks; 64.1 ± 2.3 versus $53.4 \pm 2.3\%$, 66.8 ± 3.1 versus $55.8 \pm 3.4\%$, and 69.8 ± 4.9 versus $56.1 \pm 5.4\%$ by 8, 12, and 16

weeks, respectively). The areas of type I and type II fibers reduced by 55% and 77%, respectively, at 16 weeks in the diabetic rats.

EDL muscles in the diabetic rats revealed a similar composition as in the control rats, with approximately 13% of type I versus 87% of type II fibers in both groups. Fiber areas of type I fibers were affected by diabetes with a decline of 33%, whereas type II fibers decreased by 51% at 16 weeks.

For SOL muscles, the proportion of type I was significantly higher in the diabetic rats (83.3 ± 2.3 versus $75.3 \pm 2.2\%$ by 4 weeks; 84.4 ± 3.5 versus $77.3 \pm 2.8\%$, 84.2 ± 1.4 versus $77.9 \pm 1.9\%$, and 87.5 ± 2.5 versus $79.1 \pm 2.0\%$ by 8, 12, 16 weeks, respectively).

There was a 44% reduction in areas of type I fibers and a 58% decline in areas of type II fibers for the diabetic groups at the end of the observation.

Discussion

Over the period of 16 weeks, the normal increase in body weight observed in the age-match-

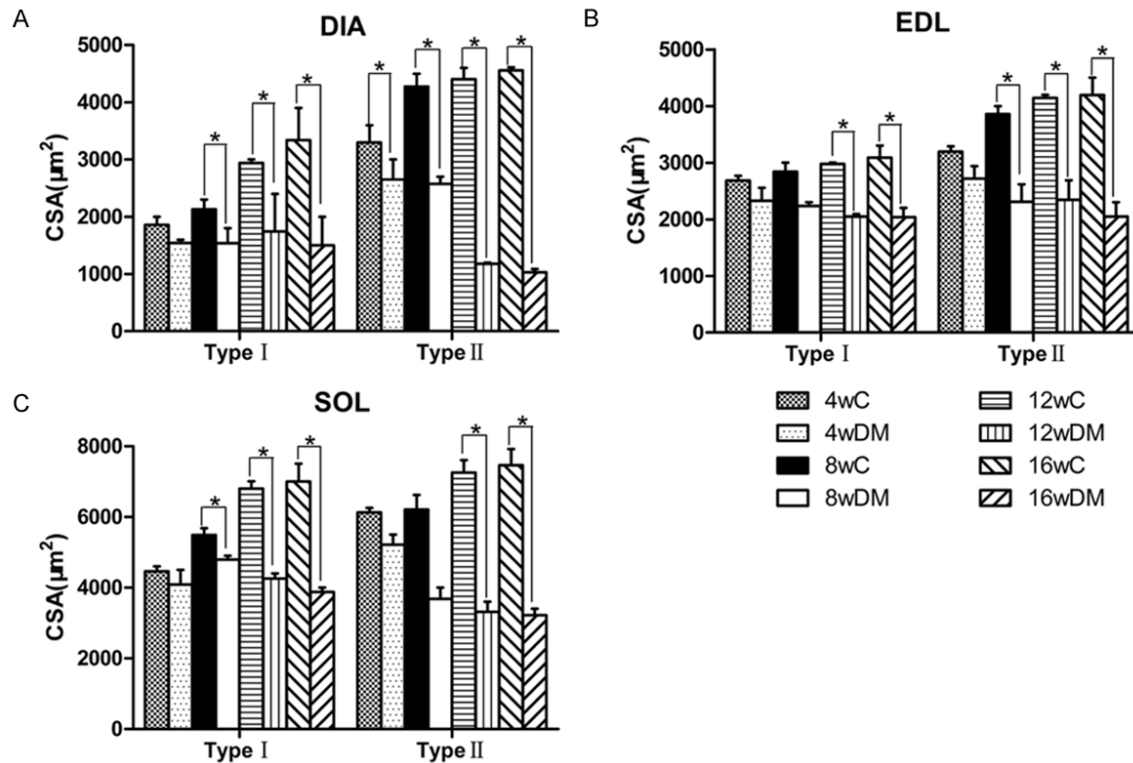


Figure 3. Fiber diameters in diaphragm (A), extensor digitorum longus (B), and soleus (C) in the diabetic and age-matched control groups. Data are presented as mean \pm SD. * $P < 0.05$ versus age-matched controls. CSA, cross-sectional area of single fiber; DIA, diaphragm; EDL, extensor digitorum longus; SOL, soleus; 4wC, control 4 weeks group; 4wDM, diabetic 4 weeks group; 8wC, control 8 weeks group; 8wDM, diabetic 8 weeks group; 12wC, control 12 weeks group; 12wDM, diabetic 12 weeks group; 16wC, control 16 weeks group; 16wDM, diabetic 16 weeks group.

ed rats was lessened by diabetes induced by STZ. Although the diabetic rats failed to maintain muscle weights of DIA, EDL, and SOL, only the M/B ratio of EDL showed a significant decrease, thus indicating preferential dystrophy of fast fibers that was reflected in fiber area determination [7]. However, the M/B ratio of DIA and SOL in the diabetic rats remained similar to the control rats. These results were consistent with the previous studies showing that fast twitch muscles were most affected by STZ-induced diabetes [7, 17]. The mechanism underlying the phenomenon is that fast fibers, especially FG fibers, are most susceptible to the catabolic action of glucocorticoids, which was testified that the corticosterone plasma level of diabetic rats rose to 134% of control rats at 33 days after STZ induction [24, 25]. Aside from the largest decline of the type II fiber area, it was established that type I fiber areas of three kinds of skeletal muscles decreased significantly, as well, which disaccorded with previous experiments [7, 26]. The discrepancy

may be due to different ages of the animals observed.

Here in this study, the twitch tensions (P_t and P_o) were normalized by CSA, and, therefore, corrected for any change in muscle size, to avoid the influence of muscle size growing larger with time going on. P_t and P_o are the values representing the capacity of contractile force, depending on the number of myosin heads bound with the actin filaments and the availability of ATP and Ca^{2+} in the sarcoplasm [27]. The fast twitch muscles are abundant in type II fibers, which have the greatest tension-producing capability, depending largely on glycogenolysis for ATP production [28]. The most affected type II fibers will lead to the decrease in P_t and P_o . Therefore, P_t and P_o of EDL will show a marked decline in diabetes. However, there are contradictory opinions about the changes of P_t and P_o in muscles of diabetic rats. In earlier reports, P_t and P_o of EDL and SOL in diabetic rats remained unchanged; P_t of DIA in diabetic

rats was similar to that of control rats, while P_0 of DIA in diabetic rats decreased [8, 29]. Nonetheless, it was shown that P_0 of EDL and SOL in diabetic rats was lower than that of controls, and P_t was of no significant decline in diabetic rats in another investigation [17]. Cotter et al demonstrated the obvious reduction of P_t and P_0 in EDL and SOL of diabetic rats compared with those of weight-matched controls [7]. Yet, in the present study, significant decrease in P_t and P_0 of DIA, EDL, and SOL in diabetic rats was observed. The findings are in agreement with the morphological results that both type I and type II fibers were affected, though type II fibers demonstrated preferential atrophy. The distinctions may be ascribed to the different control animals and the duration of diabetes. P_t/P_0 is defined as a sensitive indicator of changeover from fast to slow fibers. For SOL, the significant changes in P_t/P_0 were in accordance with the conversion in fiber types. For EDL and DIA, P_t/P_0 increased at a later stage of diabetes, indicating the later changes in muscle fiber types in these two muscles.

The prolongation of TPT and HRT was well established in the current study, which was in accordance with previous reports [7, 27, 29, 30]. The slowing of TPT and HRT may reflect the loss of fast isomyosins, and the decreases in myosin ATPase, which imply the reduced Ca^{2+} cycling ability of the SR [7, 27]. TPT represents the rapidity of the release of Ca^{2+} from the SR, and HRT means the rapidity of Ca^{2+} pump to transport Ca^{2+} from the sarcoplasm into the SR [31]. Besides the switching from fast to slow fibers verified in this investigation, the damage in myosin ATPase was reported in muscles of diabetic rats, which may result in the changes in TPT and HRT [32].

Regarding FI, ATP exhaustion rate, glycogen stores, oxidative capacity, and Ca^{2+} release depending on the cAMP-dependent stimulation of uptake and sequestering of Ca^{2+} in the SR may be relevant for the index. As for SOL and EDL, the lower oxidative capacity and higher glycogen stores of slow fibers may contribute to the resistance of SOL to fatigue in normal rats [31]. In STZ-induced diabetes, increased fatigability was documented in SOL and EDL in the current study. The higher rate of ATP exhaustion, reduced glycogen stores, increased lactate levels, higher oxidative stress, and diminished ability to utilize glycogen in muscles of

diabetic rats may account for the observation [27]. An increase in DIA was reported from 4 weeks to 8 weeks and a decline from 12 weeks to 16 weeks after STZ injection, indicating that DIA of the diabetic rats fatigued even less than that of the age-matched control rats. The interesting performance meant that adaptive changes to physiopathologic alterations, such as lack of insulin, occurred in DIA of diabetic rats at the early stage. The result of this study was in accordance with a previous study, in which a greater Ca^{2+} sensitivity of the skinned fibers at both 4 and 8 weeks after diabetes than controls was testified [11]. From 12 weeks to 16 weeks post-diabetes, the adaption was lost and increased fatigability occurred as EDL and SOL. The lower Ca^{2+} sensitivity of the contractile proteins and the loss of cooperativity between the contractile proteins in later stage of diabetes were also recorded [11]. In clinical practice, diabetic patients exhibit increased muscle fatigability and difficulty in squatting and in performing repetitive motions [33]. The differences may be relative to the character of DIA, a kind of respiratory muscles. The compensatory responses of DIA lead to greater ventilation and enhanced muscle function in the early stage of diabetes. While in the later stage, the decompensation of DIA function appears gradually to a deteriorated condition.

In conclusion, STZ-induced diabetes had differential effects on the contractile and histochemical properties of DIA, EDL, and SOL in rats. Overall, DIA, EDL, and SOL appeared to suffer from muscle atrophy in terms of lower muscle weight-to-body weight ratio and decreased muscle fiber areas. The contractile forces and speed-related properties were impaired in diabetic rats. The increased fatigability was demonstrated in DIA at a later stage, whereas resistance to fatigue in DIA at an early stage of diabetes was noted. The endurance to fatigue decreased in EDL and SOL, as well. An increased distribution of type I was observed in these muscles. The current study indicated that the differential alterations of contractile properties were associated with STZ-induced fiber transformation.

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Disclosure of conflict of interest

None.

Abbreviations

EDL, *Extensor digitorum longus muscle*; SOL, *soleus muscle*; DIA, *diaphragm*; STZ, *streptozotocin*; DM, *diabetes mellitus*; P_t , *maximal twitch force*; TPT, *time to peak tension*; HRT, *half-relaxation time*; FI, *fatigue index*; P_o , *the maximal tetanic force*; P_t/P_o , *twitch tension-to-tetanic tension ratio*; CSA, *cross-sectional area*.

Address correspondence to: Shitong Li, Department of Anesthesiology, The Affiliated First People's Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 201620, China. Tel: +86-18001780050; E-mail: honilla@163.com

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