The Effects of Long-Term Experimental Diabetes Mellitus Type I on Skeletal Muscle Regeneration Capacity

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A B S T R A C T

*Muscle fibers are dynamic structures capable of altering their phenotype under various pathological conditions. The aim of the present study was to investigate the influence of long-lasting diabetes mellitus on the process of muscle regeneration in the skeletal muscle. Wistar rats were made diabetic by a single intraperitoneal injection of streptozotocin (STZ). The regeneration process in the skeletal muscle was induced in slow (*m. soleus*, SOL) and fast (*m. extensor digitorum longus*, EDL) muscles by injection of local anesthetic (bupivacaine). Skeletal muscles were analyzed 10 days, 4 and 8 weeks after bupivacaine treatment. Diabetes mellitus has changed morphological properties of both slow and fast skeletal muscles during the process of regeneration. These changes are evident in redistribution of muscle fibers and significant level of atrophy. All fiber types of diabetic fast muscles showed stronger atrophy than muscle fibers in slow muscles which have more oxidative metabolism. The changes of redistribution of muscle fibers depend on duration of diabetes and affect all types of muscle fibers.*

Key words: skeletal muscle, regeneration, type 1 diabetes mellitus, atrophy, rat

Introduction

Streptozotocin (STZ)-induced diabetes mellitus type 1 is the most commonly used animal model for the study of human diabetes mellitus. This model has provided useful information about physiological and pathological changes produced in skeletal muscle by experimental diabetes. Insulin is a major anabolic hormone in the body, and its deficiency has effects on many processes and leads to general disturbance of metabolism. The skeletal muscle is the main site of post absorptive glucose disposal and a major target of insulin action, both on glucose uptake and blood flow[1]. The skeletal muscle is composed of a heterogeneous population of muscle fibers which express different myosin heavy chain (MHC) isoforms[2]. Four different types of muscle fibers with different physiological properties can be identified using specific monoclonal antibodies to MHC isoforms: slow-contracting fibers, called type I, and three different types of fast-contracting fibers, called IIA, IIX and IIB muscle fibers[3,4]. It has been well documented that the skeletal muscle, the major target of insulin action, changes its histochemical and morphometrical characteristics during the progression of diabetes[5–7]. These effects on the skeletal muscle were considered to be formed by a combination of neurogenic and myogenic factors[8,9]. It has been demonstrated that experimentally induced diabetes mellitus type 1 in the rat is characterized by a considerable disturbance of muscle protein turnover[10], a decrease of protein synthesis[11] and an increase in protein degradation[12]. Skeletal muscle fibers have a remarkable ability to complete a rapid and extensive regeneration[13]. Experimentally induced skeletal muscle regeneration is a very good model of myogenesis, which can be used to study muscle gene expression and remodeling of the contractile apparatus under various pathological conditions such as insulin de-

ficiency. Induced muscle regeneration is characterized by two phases: a degenerative phase with muscle necrosis and a regenerative phase with muscle fiber differentiation which takes place rapidly and synchronously[3,14]. The aim of the present time-course study was to investigate the influence of the diabetes mellitus type 1 on the process of muscle regeneration in fast and slow skeletal muscles.

Materials and Methods

Experimental design

Male Wistar rats weighing 260–310 g, two months old, were randomly assigned to STZ-diabetic or control group, maintained on a 12-hour dark and light schedule and fed according to appetite with Purina rat chow. After overnight fast, animals were anaesthetized with ether, and streptozotocin (STZ, Zanosar, Pharmacia-UpJohn, USA) was given by a single intraperitoneal injection (65/mg/kg). Two days later blood glucose concentration was measured with glucose monitor (Accu-Chek II, Roche Diagnostic, Germany). The criterion for diagnosing an animal as diabetic was a blood glucose concentration greater than 16.5 mmol/L. After the diagnosis of diabetes, the regeneration process was induced in slow (*m. soleus*, SOL) and fast (*m. extensor digitorum longus*, EDL) skeletal muscles by injection of local anesthetic, bupivacaine.

Blood glucose concentration and body weights of animals were recorded at least once a week. At intervals of 10 days, 4 and 8 weeks, diabetic animals and their age-matched control animals were sacrificed by overdose of anesthesia. Blood samples (collected into lithium-heparin anticoagulant containers) were centrifuged at 4°C. The muscles were removed and frozen in liquid nitrogen and stored at –80°C until further analysis. Serial transverse 10 μm-thick sections were cut on a cryomicrotome, dried at room temperature and preceded for immunohistochemistry.

Immunohistochemistry for MHC

Transverse cryosections of SOL and EDL muscles were stained for slow and fast myosin heavy chain isoforms using monoclonal antibodies specific for fiber type I (BF-F8), IIA (SC-71), IIB (BF-F3) and for fibre type IIX (BF-35). The antibodies were given by Prof. Stefano Schiaffino, Department of Biomedical Sciences, Padua, Italy. The slides were incubated at room temperature with monoclonal antibodies (1:1000) in phosphate-buffered saline (PBS) for 30 minutes. After three wash steps in PBS, the slides were incubated for 30 minutes with peroxidase-labelled rabbit anti-mouse IgG (Dako A/S, Copenhagen, Denmark) in PBS (1:40). After washing in PBS, the slides were incubated in 50 mL of diamino-benzidine (DAB) solution (0.5M tris HCl, pH 7.6, 15 mg of DAB, 100 μL of H_2O_2, 25 m of imidazol) for 20 minutes at room temperature. The slides were dried and mounted in Canada balsam.

Fiber typing and morphometry

The fiber type frequencies and cross sectional areas were analyzed by the computer program for quantitative analysis »SFORM« (VAMS, Zagreb, Croatia). One hundred fibers of each type were measured by moving a pen along the circumference of the fibers. The mean fiber size with standard deviation (SD) was calculated. Control groups were used for comparison with denervated muscles. Statistical evaluations were performed by Student t-test at $p < 0.001$ level of significance.

Statistical analysis

Data are expressed as means±SE. The differences among experimental groups were determined by analysis of variance, and the significance of the differences between these groups was assessed by the Student-Newman-Keuls multiple range test.

Results

Baseline body weights, plasma glucose level and glycosylated hemoglobin are summarized in Table 1. The untreated diabetic animals exhibited a significant weight loss during the four-week and eight-week study period. As expected, the untreated diabetic rats had marked hyperglycemia and elevated glycosylated hemoglobin in respect to control animals.

TABLE 1

BODY WEIGHT, PLASMA CONCENTRATIONS OF GLUCOSE AND GLYCOSYLATED HEMOGLOBIN (HbA1C) IN CONTROL RATS FED REGULAR DIET AND UNTREATED DIABETIC RATS

	Controls			Diabetes	Mellitus	
	10 days	Week 4	Week 8	10 days	Week 4	Week 8
Body weight (g)	310±14.1	370±14.1	380±56.6	285.7±39.9*	233±40.4*	249.5±37.7*
Plasma glucose (mmol/l)	5.8±0.2	5.1±0.7	5.9±0.4	29.1±1.4*	32.3±0.9*	30.0±0.9*
HbA1C (%)	2.3±0.14	3.05±0.07	2.8±0.14	4.17±0.19*	6.17±0.06*	6.53±0.81*

Data are $\overline{X}$±SE. * $p < 0.001$ *vs*. control.

Fibre type distribution of soleus and extensor digitorum longus muscle

The fibre type distribution of control and diabetic SOL muscle is demonstrated in Figure 1. The soleus muscle of control rats is a slow twitch muscle which is composed of 87–95% fibers of type I and 5–11% fibers of type IIA. As far as diabetic muscles are concerned, there were no significant changes in fiber type distribution. The most evident finding in diabetic SOL muscle was the complete absence of type IIA fibers, i.e., in the 8th week of muscle regeneration type I fibers became dominant.

The fibre type distribution of control and diabetic EDL muscle is demonstrated in Figure 2. *Extensor digitorum longus* as a fast twitch muscle is composed of 6–13% fibers of type I, of 28–41% fibers of type IIA, of 0–45% fibers of type IIX and of 21–58% fibers of type IIB. After 8 weeks of regeneration, the diabetic muscles showed significantly higher percentage of type I and type IIA fibers, which have predominant oxidative metabolism.

Fibres cross areas in soleus *and* extensor digitorum longus *muscle*

Diabetic SOL muscles show considerable differences in fibre cross areas in respect to control muscles (Figure 3). Fiber cross area of diabetic soleus IIA muscle fibers was significantly smaller than in control muscle at the stage of 10 days and 4 weeks of diabetes mellitus. As shown previously, after 8 weeks of diabetes type IIA fibres completely disappeared. Moreover, it was evident that, parallel to the disappearance of type IIA muscle fibers, the diameter of type I muscle fibers has increased.

Diabetic EDL muscles show considerable differences in fibre cross areas in respect to control EDL muscles (Figure 4). In diabetic EDL muscles, the cross areas of all fibre types were significantly smaller in respect to control muscles at all stages of the regeneration process. It is interesting to notice that after 10 days of duration of diabetes there were no type IIX fibres.
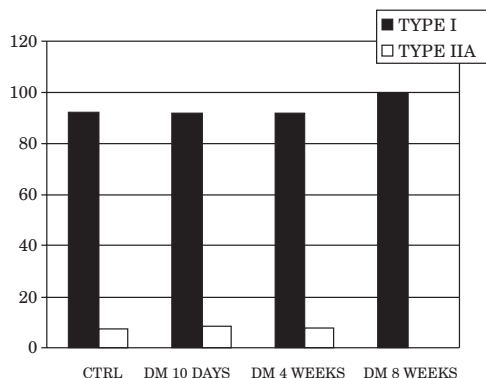

Fig. 1. Changes in fibre type distribution of rat soleus muscle in control group (CTRL) and in groups with streptozocin induced diabetes mellitus type 1 after the period of 10 days (DM 10 DAYS), 4 (DM 4 WEEKS) and 8 weeks (DM 8 WEEKS).

Fig. 3. Fibre cross area (μm^2) of rat soleus muscle in control group (CTRL) and in diabetic animals during the period of 10 days (DM 10 DAYS), 4 weeks (DM 4 WEEKS) and 8 weeks (DM 8 WEEKS).
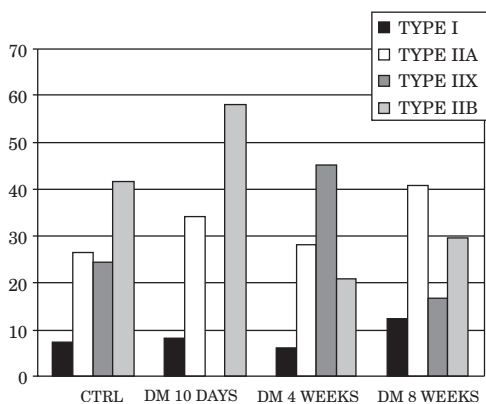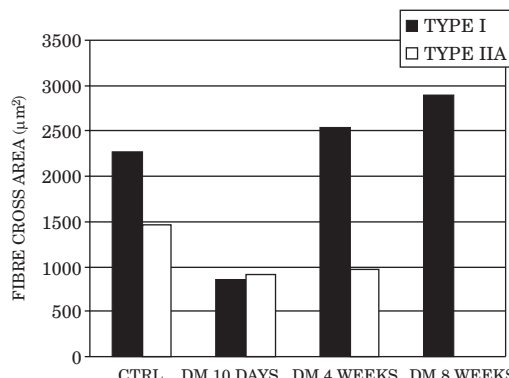

Fig. 2. Changes in fibre type distribution of rat extensor digitorum muscle (EDL) in control group (CTRL) and in groups with streptozocin induced diabetes mellitus type 1 after the period of 10 days (DM 10 DAYS), 4 (DM 4 WEEKS) and 8 weeks (DM 8 WEEKS).

Fig. 4. Fibre cross area ($\mu m2$) of rat extensor digitorum muscle in control group (CTRL) and in diabetic animals during the period of 10 days (DM 10 DAYS), 4 weeks (DM 4 WEEKS) and 8 weeks (DM 8 WEEKS).
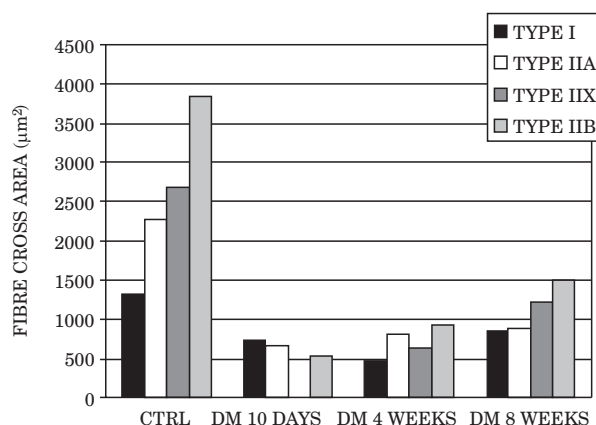
Discussion

We have investigated the process of skeletal muscle regeneration of slow and fast muscles in streptozotocin--induced diabetes mellitus type 1 during the muscle regeneration process induced by intramuscular injection of myotoxin bupivacaine. This model of muscle regeneration is characterized by inflammatory response and mononuclear cell proliferation within 1–4 days of bupivacaine injection. Myogenic cell differentiation and new myotube formation was observed 5–6 days post injection. By day 10 the muscle architecture was restored, but myofibers were smaller and displayed central myonuclei. The normal mature muscle was recovered at 3–4 weeks post injection. All the time, the vessels, satellite cells and nerves were intact[15].

Soleus, which is mainly a slow muscle, is composed dominantly of type I fibers and of a small percentage of IIA muscle fibers. It is well known that diabetes induces neuropathy, and changes in fiber type distribution can be a response to this damage. In this study we have shown that after 8 weeks of diabetes there were no type IIA fibers. This might be an age-related alteration in muscle tissue which is commonly seen in the process of ageing skeletal muscle[16,17]. As far as fiber diameter is concerned, it has demonstrated the reduction of IIA fiber area until the disappearance of this fiber type. It has also shown concomitant enlargement of type I cross-sectional area. Diabetes mellitus type 1 has caused changes in fibre type distribution and a significant level of atrophy of EDL muscle. Our results have demonstrated that after 4 weeks

of diabetes, type IIX muscle fibers are predominant muscle fibers, but after 8 weeks of diabetes there are more fibers with oxidative metabolism (type I and IIA). The most important and most evident finding of our study is a strong atrophy of all muscle fibers in EDL muscle. Skeletal muscle exhibits a high degree of plasticity, adapting its phenotype expression to its metabolic profile. It has already been shown that for example ageing and hypoxia can change skeletal muscle phenotype[18,19]. The level of atrophy of fast regenerating muscles in diabetes mellitus is larger than in slow muscles. Our study of regeneration capacity of fast diabetic muscles shows similar results to those previously obtained[9,20,21].

Conclusion

We have found that diabetes mellitus type 1 cause the morphological properties in both slow and fast muscles during the muscle regeneration process. These changes are evident in the redistribution of muscle fibers and in the significant level of atrophy. Diabetic extensor digitorum longus which represents fast skeletal muscle has shown much stronger atrophy of all muscle fibers in respect to control muscles. The changes of redistribution of muscle fibers and the degree of atrophy depend on the duration of experimentally induced diabetes mellitus type 1.

The long lasting diabetes caused shift to more oxidative metabolism of skeletal muscle, which is commonly seen in the process of ageing of skeletal muscle.

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UTJECAJ EKSPERIMENTALNO IZAZVANE ŠEĆERNE BOLESTI TIP 1 NA REGENERACIJSKU SPOSOBNOST SKELETNOG MIŠIĆA

S A Ž E T A K

Mišićna vlakna su dinamične strukture sposobne mijenjati svoj fenotip u različitim patološkim stanjima. Cilj ove studije je bio istražiti utjecaj dugotrajne šećerne bolesti na proces regeneracije skeletnog mišića. Šećerna bolest tip 1 u Wistar štakora izazvana je intraperitonealnom injekcijom streptozocina. U sporom (*m. soleus*) i brzom skeletnom mišiću (*m. extensor digitorum longus*) je izazvan proces regeneracije intramuskularnom injekcijom lokalnog anestetika (bupivacain) Mišići dijabetičnih štakora analizirani su nakon 10 dana, 4 i 8 tjedana od tretmana bupivakainom. Šećerna bolest uzrokovala je promjene morfoloških karakteristika kako sporog tako i brzog skeletnog mišića tijekom procesa regeneracije. Ove promjene se odražavaju u izmjenjenoj zastupljenosti pojedinih tipova vlakana u mišiću kao i u značajnom stupnju atrofije. Svi tipovi mišićnih vlakana u brzom skeletnom mišiću u šećernoj bolesti pokazuju jači stupanj atrofije od tipova mišićnih vlakana u sporom mišiću koja imaju pretežno oksidativni metabolizam. Promjene u zastupljenosti pojedinih tipova vlakana u mišiću ovise o dužini trajanja šećerne bolesti i zahvaćaju sve tipove mišićnih vlakana, ali u mnogo većoj mjeri brza mišićna vlakna.