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GENDER AND CONTRACTILE FUNCTIONS OF SLOW AND FAST SKELETAL MUSCLES IN STREPTOZOTOCIN INDUCED DIABETIC SPRAGUE DAWLEY RATS

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Abstract

Objectives: *Diabetes mellitus has been linked with specific morphological and metabolic abnormalities of skeletal muscle in a fiber specific manner.* ***Aim:*** *The present study was designed to compare the contractile functions of slow and fast skeletal muscles in streptozotocin (STZ) induced diabetic male and female Sprague Dawley rats.* ***Material and methods:*** *Thirty healthy Sprague Dawley rats (15 male and 15 female) were divided into two groups and studied after four weeks following diabetes induction. The rats in group I (male diabetic; n = 15) and group II (female diabetic; n = 15) were fed on normal pellet diet and water ad libitum and rendered diabetic by single intraperitoneal injection of STZ 65 mg/kg body weight at the start of study (day 1). At the end of four weeks, the contractile parameters of slow soleus and fast extensor digitorum longus (EDL) muscles were recorded by iWorx advanced animal/human physiology data acquisition unit (AHK/214).* ***Results:*** *At the end of four weeks, the weight of isolated soleus and EDL muscles in the male diabetic rats was significantly higher ($p < 0.001$) as compared to the female diabetic rats. However, no significant difference was found in any of the contractile functions of isolated soleus and EDL muscles when compared between the male and female diabetic rats.* ***Conclusion:*** *No gender differences exist in the contractile functions of slow and fast skeletal muscles in streptozotocin induced diabetic Sprague Dawley rats.*

key words: *Streptozotocin, diabetes mellitus, blood glucose, soleus, extensor digitorum longus*

Background

Diabetes mellitus has been linked with specific morphological and metabolic abnormalities of skeletal muscle in a fiber specific manner. A more rapid down regulation of GLUT4 glucose transporter protein and mRNA expression has been observed in slow-twitch or type I muscle fibers as compared to the fast-twitch or type II fibers [1]. Furthermore, in type 1 diabetes (T1DM), slow-twitch oxidative or type I fibers

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have shown a greater accumulation of intramyocellular lipids (IMCL) [2]. On the other hand, studies have shown that type IIB or fast-twitch glycolytic fibers undergo the most severe atrophy due to the oxidative stress mediated by hyperglycemia (e.g., production of advanced glycation end products and reactive oxygen species). The rate of protein degradation is much greater in type IIB fibers as compared to type I fibers with a parallel decline in protein synthesis [3]. It has been shown that several other hormones such as glucocorticoids, growth hormone (GH), plasminogen activator inhibitor-1 (PAI-1) and insulin-like growth factor-1 (IGF-1) affect skeletal muscle contractile functions in T1DM [4].

Much of our current knowledge regarding diabetic myopathy is the result of studies conducted on adult streptozotocin (STZ) induced diabetic rodents. The basic indices of skeletal muscle phenotype and function, such as fiber type composition, fiber size, intramyocellular lipid content (IMCL), capillary-to-fiber ratio, and contractile parameters, have all been demonstrated to be altered in the STZ induced diabetic model [5]. Streptozotocin (STZ; N-nitro derivative of glucosamine) is a naturally occurring, broad spectrum antibiotic and cytotoxic chemical that is specifically toxic to the pancreatic, insulin secreting β cells in mammals [6]. Clinically, symptoms of diabetes are clearly seen in rats within 2-4 days following single intravenous or intraperitoneal injection of 60 mg/kg STZ [7]. It has been documented that STZ decreases insulin biosynthesis and secretion. STZ generates nitric oxide intracellularly, which causes alkylation and fragmentation of deoxyribonucleic acid (DNA). STZ also generates reactive oxygen species (ROS), which contribute to DNA fragmentation [8].

To our knowledge, no study to date has explored the effect of gender on the contractile functions of slow and fast skeletal muscles in T1DM. It has been well documented that the body mass of male rats is approximately two times higher than that of female rats [9]. A number of previous human studies have shown that the males develop larger specific force as compared to the females but others have found no differences [10,11]. Furthermore, gender based differences in the kinetics of force production and fatigue resistance in skeletal muscles have been frequently reported. It has been shown that the females generally exhibit relatively higher resistance to fatigue as compared to the males due to the differences in blood supply to the working muscles, higher percentage of type I, slow-twitch, fatigue resistant fibers, and greater oxidative capacity under conditions mimicking T1DM e.g., caloric restriction [12]. T1DM is characterized by early fatigue of skeletal muscles on exertion which leads to reduced physical activity of diabetic patients over time [13]. Therefore, an understanding of the gender based differences in slow and fast skeletal muscle functional decline in T1DM is very important. In view of the above, the present study was designed to compare skeletal muscle functions, that is, isometric contraction, force-frequency relationship, and fatigue in slow soleus and fast extensor digitorum longus (EDL) muscles in STZ induced diabetic male and female Sprague Dawley rats. These muscles were selected to investigate the contractile functions because they represent two different fiber type

populations. The slow soleus muscle is mainly comprised of type I or slow-twitch oxidative fibers whereas the fast extensor digitorum longus (EDL) muscle is mainly comprised of type II or fast-twitch glycolytic fibers [14].

Material and methods

Thirty healthy Sprague Dawley rats (15 male and 15 female), 80 ± 5 days old and weighing 200-300 grams, were divided into two groups. The rats in group I (male diabetic; n = 15) and group II (female diabetic; n = 15) were rendered diabetic by single intraperitoneal injection of 65mg/kg STZ (Bioplus) in normal saline at the start of study (day 1) [13]. Development of diabetes was confirmed within 72 hours, by the measurement of blood glucose levels by glucometer (blood glucose $\geq$ 200 mg/dl) [15]. Blood glucose was measured at regular intervals after every week, throughout the study, until the completion of study 4 weeks later (day 29). Rats were fed on normal pellet diet and water *ad libitum*.

At the end of four weeks, the rats were anaesthetized by administering single intraperitoneal injection of sodium pentobarbitone (50 mg/100 g body weight) [16]. The soleus and EDL muscles were dissected free from the surrounding connective tissue [17,18] and weighed on an electronic balance. For the measurement of contractile functions, the muscles were mounted in an organ bath containing Krebs-Ringer solution, gassed with 95% O_2 – 5% CO_2 at 30°C. The proximal tendons of isolated soleus and EDL muscles were alternatively tied to the force transducer (FT-100) connected to iWorx advanced animal/human physiology data acquisition unit (AHK/214). Contractions were evoked by stimulation via platinum electrodes placed directly on to the muscle. Labscribe® software was used to collect, digitize, analyze, and store the data to a personal computer. The length of each muscle was adjusted for maximal twitch tension. Passive and twitch tensions were then recorded. The speed related contractile properties were monitored by measuring time to peak twitch tension and time taken to relax to 50% of the peak twitch tension. The force-frequency relationship was determined by using stimulations of 1 second. Stimulation frequencies of 5-90 Hz for the isolated soleus muscle and 5-110 Hz for the isolated EDL muscle were used. Rest period of 3 minutes was allowed between each stimulus. The maximum fused tetanic tension was then recorded. The fatigue characteristics of each muscle were determined by stimulating the muscle with optimum frequency for 1 second with 5 seconds rest period in between, for a total period of 5 minutes. A measure of recovery from fatigue was also made by recording the tetanic tension after a 5 minutes rest period following the fatigue protocol [19]. All measured forces were expressed as Newton per gram (N/g) wet muscle mass [20].

Data analysis

Data was entered into SPSS version 18. Mean and standard deviation was calculated for skeletal muscle function variables. The statistical significance of difference between the groups was determined by applying independent sample's t-test. The difference was considered significant if p-value was found less than 0.05.

Results

At the end of four weeks of study, that is, on day 29, blood glucose level in the male diabetic rats (302.67 ± 4.89 mg/dl) was significantly higher ($p < 0.001$) as compared to the baseline blood glucose level (93.47 ± 3.00 mg/dl) before induction of diabetes. Similarly, blood glucose level in the female diabetic rats (233.27 ± 6.17 mg/dl) was significantly higher ($p < 0.001$) as compared to the baseline blood glucose level (67.07 ± 4.73 mg/dl) before induction of diabetes mellitus.

The comparison of weight of isolated soleus and EDL muscles between the male and female diabetic rats is given in Figure 1. The weight of isolated soleus and EDL muscle in the male diabetic rats was significantly higher ($p < 0.001$) as compared to the female diabetic rats.

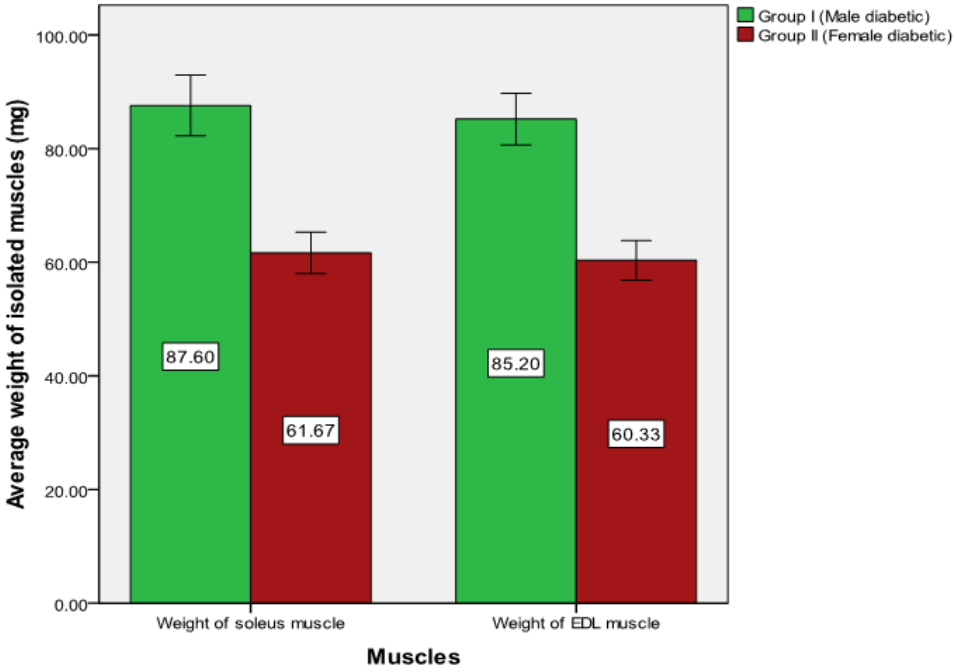

Figure 1. Comparison of weight of isolated soleus and extensor digitorum longus (EDL) muscles between diabetic female and male Sprague Dawley rats at the end of four weeks. $p < 0.001$; mean muscle weight of female diabetic rats *vs.* male diabetic rats.

The normalized contractile properties of isolated soleus and EDL muscles in the male and female diabetic groups are given in Tables 1 and 2.

The maximum isometric twitch tension was similar between the male and female diabetic rats in isolated soleus ($p = 0.086$) and EDL muscles ($p = 0.095$). No significant difference was observed in time to peak twitch tension in isolated soleus ($p = 0.078$) and EDL muscles ($p = 0.370$) between the male and female diabetic rats. Similarly, no significant difference was observed in the time taken to relax to 50% of the peak twitch tension in isolated soleus ($p = 0.073$) and EDL muscles ($p = 0.409$) between the male and female diabetic rats.

The maximum fused tetanic tension was similar in isolated soleus ($p = 0.765$) and EDL muscles ($p = 0.550$) between the male and female diabetic rats. Similarly, maximum fused tetanic tension after the fatigue protocol

was similar in isolated soleus (p = 0.083) and EDL muscles (p = 0.100) between the male and female diabetic rats. No significant difference was observed in tetanic tension after 5 minutes of rest period following the fatigue protocol in isolated soleus (p = 0.068) and EDL muscles (p = 0.167) between the male and female diabetic rats.

Table 1. Comparison of normalized contractile properties of isolated soleus muscle between diabetic female and male Sprague Dawley rats at the end of four weeks of study.

Contractile properties of soleus muscle	Group I (Male diabetic) n = 15	Group II (Female diabetic) n = 15	p-value
Maximum isometric twitch tension (N/g)	1.404 ± 0.032	1.385 ± 0.029	0.086
Time to peak twitch tension (ms)	21.85 ± 1.23	21.05 ± 1.17	0.078
Time taken to relax to 50% of the peak twitch tension (ms)	33.19 ± 4.14	30.50 ± 3.76	0.073
Maximum fused tetanic tension (N/g)	8.95 ± 0.481	8.99 ± 0.371	0.765
Maximum fused tetanic tension after the fatigue protocol (N/g)	5.56 ± 0.619	5.92 ± 0.485	0.083
Tetanic tension after 5 minutes of rest period following the fatigue protocol (N/g)	6.49 ± 0.491	6.84 ± 0.525	0.068

All values have been expressed as mean ± SD

Table 2. Comparison of normalized contractile properties of isolated extensor digitorum longus (EDL) muscle between diabetic female and male Sprague Dawley rats at the end of four weeks of study.

Contractile properties of EDL muscle	Group I (Male diabetic) n = 15	Group II (Female diabetic) n = 15	p-value
Maximum isometric twitch tension (N/g)	1.378 ± 0.025	1.394 ± 0.026	0.095
Time to peak twitch tension (ms)	7.94 ± 0.138	7.98 ± 0.109	0.370
Time taken to relax to 50% of the peak twitch tension (ms)	6.91 ± 0.154	6.96 ± 0.142	0.409
Maximum fused tetanic tension (N/g)	11.46 ± 0.846	11.64 ± 0.782	0.550
Maximum fused tetanic tension after the fatigue protocol (N/g)	2.87 ± 0.068	2.92 ± 0.070	0.100
Tetanic tension after 5 minutes of rest period following the fatigue protocol (N/g)	7.91 ± 0.786	8.35 ± 0.884	0.167

All values have been expressed as mean ± SD

Discussion

Gender differences in skeletal muscle cross-sectional area, muscle fiber type and number have been shown to occur in various species including humans, rats, and mice, however, evidence for the effect of gender on muscle contractile properties has been equivocal [21]. The results of our study indirectly support the finding that similar level of damage is caused by diabetes in skeletal muscles of both male and female rats. A study conducted on Sprague Dawley rats revealed that 14 days of alloxan-induced diabetes resulted in similar degree of oxidative stress in skeletal muscles of both male and female rats despite marked changes in hormonal, physiological and biochemical parameters in

male and female rats. Alloxan-induced diabetes caused an elevation in lipid peroxidation, cholesterol and triglyceride concentrations, but resulted in a decrease in catalase activity in the liver and kidney of both male and female rats. Data of that study indicated that gender difference did not significantly affect oxidative stress in alloxan-induced diabetes [22].

The present study is the first gender based study, designed to compare the contractile functions of slow and fast skeletal muscles in streptozotocin (STZ) induced diabetes in Sprague Dawley rats. An animal model of STZ induced diabetes was used in the present study owing to its convenience [23]. The dose of STZ used in the present study (65 mg/kg body weight) has been associated with minimum risk of mortality in the experimental rodents [24]. Previous studies have employed similar animal models of STZ induced diabetes [13,25].

At the end of four weeks of study, the weight of isolated soleus and EDL muscles in the male diabetic group was significantly higher ($p < 0.001$) as compared to the female diabetic group. Previous studies have shown that STZ induced diabetes leads to decreased number and diameter of both slow and fast skeletal muscle fibers in rats irrespective of their gender [26]. Therefore, the significant difference in the weight of isolated soleus and EDL muscles between the male and female diabetic rats reflects the gender based difference in the muscle mass rather than a differential effect of diabetes on the skeletal muscle fibers of male and female rats. Similar significant difference in weight of soleus, biceps brachii and sternomastoideus muscles between the diabetic male and female Sprague Dawley rats has been previously reported [26].

The greater mass of isolated soleus and EDL muscles of the male diabetic rats of our study resulted in significantly greater force production and higher values of absolute twitch and tetanic tensions as compared to the female diabetic rats. However, when the twitch and tetanic tensions were normalized to per gram of muscle mass, no significant difference was found in specific force or any other contractile parameter of isolated soleus and EDL muscles between the male and female diabetic rats. In another study conducted on young healthy Wistar rats, no significant difference was observed in specific force or any other contractile parameter of geniohyoid and sternohyoid muscles of male and female rats, supporting the finding that no gender difference exists in the contractile properties of muscles [27].

Previous studies have shown that the liver and brown adipose tissue of the female rats have higher capacity of oxidative metabolism as compared to the male rats which could be due to their higher mitochondrial content and biogenesis [28,29]. In another study, gastrocnemius muscle of the female rats showed higher mitochondrial DNA and protein contents, mitochondrial transcription factor A (TFAM) protein level, oxidative and phosphorylative machinery and activities, and glutathione peroxidase activity as compared to the male rats during three months of caloric restriction [30]. However, in the present study, the magnitude of such differences was probably not sufficient to effect the whole muscle contractile measurements between the male and female diabetic Sprague Dawley rats.

In concluding the observations of our present study, it must be kept in mind that the relationship of fiber type and contraction is different in rat muscle when compared to human skeletal muscle and effect of streptozotocin on skeletal muscle cellularity must be evaluated with caution [31].

Conclusion

It is concluded that no gender differences exist in the contractile functions of slow and fast skeletal muscles in STZ induced diabetic Sprague Dawley rats.

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