Original Article

CONTRACTILE FUNCTIONS OF SLOW AND FAST SKELETAL MUSCLES IN STREPTOZOTOCIN INDUCED TYPE 1 DIABETIC SPRAGUE DAWLEY RATS

Kamil Asghar Imam, Muhamamd Mazhar Hussain, Shoaib Bin Aleem

Army Medical College Rawalpindi

ABSTRACT

Objective: To evaluate the contractile functions of slow and fast skeletal muscles in streptozotocin induced type 1 diabetic male Sprague Dawley rats.

Study Design: Randomized control trial.

Place and Duration: Department of Physiology, Army Medical College, Rawalpindi, from April 2010 to April 2011.

Material and Methods: Thirty healthy male Sprague Dawley rats were divided into two groups. The rats in group I (male control; n = 15) were fed on normal pellet diet and water ad libitum and received single intraperitoneal injection of normal saline at the start of study (day 1). The rats in group II (male diabetic; n = 15) were fed on normal pellet diet and water ad libitum and rendered diabetic by single intraperitoneal injection of streptozotocin (STZ) 65 mg/kg body weight at the start of study (day 1). Development of diabetes was confirmed within 72 hours by measuring blood glucose levels by glucometer. At the end of four weeks, i.e on day 29, dissection of slow soleus and fast extensor digitorum longus (EDL) muscles was carried out. These muscles were selected because they represent two distinctly different fiber type populations, that is, soleus (80% type I, 20% type IIA, 0% type IIB) and EDL (0% type I, 11% type IIA, 89% type IIB). Their contractile parameters were recorded by iWorx advanced animal/human physiology data acquisition unit (AHK/214), including maximum isometric twitch tension, time to peak twitch tension, time taken to relax to 50% of the peak twitch tension, maximum fused tetanic tension, maximum fused tetanic tension after the fatigue protocol and tetanic tension after 5 minutes of rest period following the fatigue protocol.

Results: After four weeks, no significant difference was found when maximum isometric twitch tension (ITT) in isolated soleus and EDL muscles of the male diabetic group was compared with the control group. Time to peak twitch tension (TPT) and time taken to relax to 50% of the peak twitch tension (HRT) in isolated soleus muscle of the male diabetic group were significantly longer (p<0.001) as compared to the control group. On the contrary, TPT and HRT in isolated EDL muscle of the diabetic group were similar to the control group. Maximum fused tetanic tension in isolated soleus muscle of the diabetic group was similar to the control group. On the contrary, maximum fused tetanic tension in isolated EDL muscle of the male diabetic group was significantly lower (p<0.001) as compared to the control group. Maximum fused tetanic tension after the fatigue protocol and tetanic tension after 5 minutes of rest period following the fatigue protocol in isolated soleus and EDL muscles of the male diabetic group were significantly lower (p<0.001) as compared to the control group.

Conclusion: Streptozotocin induced type 1 diabetes mellitus manifests differential effects on the contractile properties of slow and fast skeletal muscles of male Sprague Dawley rats.

Keywords: Streptozotocin, type 1 diabetes mellitus, blood glucose, soleus, extensor digitorum longus.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is associated with specific morphological and metabolic abnormalities of skeletal muscle in a fiber specific fashion. 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, slow-twitch oxidative or type I fibers 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

Correspondence: Major Kamil Asghar Imam, Physiology Departmetn, Army Medical College Rawalpindi
Email: kkamzz@hotmail.com
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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].

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[4]. 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. STZ action on the mitochondria inhibits the citric acid cycle and significantly decreases oxygen consumption by mitochondria. This strongly inhibits mitochondrial ATP production and causes its depletion. Depletion of ATP inhibits insulin synthesis and secretion. All these factors lead to the destruction of β cells of pancreas and also decreased insulin secreting capacity of the remaining cells[5].

Increased fatigability of skeletal muscles characterized by difficulty in performing repetitive movements is commonly observed in T1DM. An increased susceptibility to muscle fatigue reinforces the tendency to physical inactivity typical of the diabetic patient[6]. Therefore, an understanding of slow and fast skeletal muscle functional decline in T1DM is very important. In view of the above, the present study was designed to evaluate skeletal muscle contractile functions, that is, isometric contraction, force-frequency relationship, and fatigue in slow soleus and fast extensor digitorum longus (EDL) muscles in streptozotocin induced type 1 diabetic male Sprague Dawley rats. These muscles were selected to record 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[7].

MATERIAL AND METHODS

These randomized control trials were carried out in the Department of Physiology, Army Medical College, Rawalpindi from April 2010 to April 2011. Thirty healthy male Sprague Dawley rats, 80 ± 5 days old and weighing 200-300 grams, were randomly divided into two groups. The rats in group I (male control; n = 15) received single intraperitoneal injection of normal saline at the start of study (day 1). The rats in group II (male diabetic; n = 15) were rendered diabetic by single intraperitoneal injection of 65mg/kg streptozotocin (Bioplus) in normal saline at the start of study (day 1)[6]. Development of diabetes was confirmed within 72 hours, by the measurement of blood glucose levels by glucometer (blood glucose > 200 mg/dl)[8]. Blood glucose was measured at regular intervals after every week, throughout the study, until the completion of study 4 weeks later (day 29). The rats in group I and group II 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)[9]. The soleus and extensor digitorum longus (EDL) muscles were dissected free from the surrounding connective tissue[10,11]. For the measurement of contractile functions, the muscles were mounted in an organ bath containing Krebs-Ringer solution, gassed with 95% O2 – 5% CO2 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 the total period of 5 minutes. A measure of recovery from fatigue was also made by recording the tetanic tension after the 5 minutes rest period following the fatigue protocol[12]. All measured forces were expressed as Newton per gram (N/g) wet muscle mass[13].

Data Analysis

Data was entered into SPSS version 18. Mean and standard deviation (SD) were 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 group (302.67 ± 4.89 mg/dl) was significantly higher ($p < 0.001$) as compared to the male control group (113.47 ± 4.07 mg/dl). The contractile properties of isolated soleus muscle in the male diabetic rats and healthy controls have been compared in table 1. No significant difference was found between cases and controls in maximum isometric twitch tension (p=0.153) and maximum fused tetanic tension (p=0.126). Time to peak twitch tension (TPT) and time taken to relax to 50% of the peak twitch tension (HRT) in isolated soleus muscle of the male diabetic group was significantly longer (p<0.001) as compared to the male control group. Maximum fused tetanic tension after the fatigue protocol in isolated soleus muscle of the male diabetic group was significantly less ($p < 0.001$) as compared to the male control group. Similarly, tetanic tension after 5 minutes of rest period following the fatigue protocol in isolated soleus muscle of the male diabetic group was significantly lower (p<0.001) as compared to the male control group.

The contractile properties of isolated extensor digitorum longus (EDL) muscle in male diabetic rats and healthy controls have been compared in table 2. Isometric twitch tension (ITT) in isolated extensor digitorum longus (EDL) muscle of the male diabetic and control groups was similar (p=0.062) with no significant difference. Time to peak twitch tension (TPT) in isolated EDL muscle of the male diabetic group was similar (p=0.342) to the male control group. Similarly, time taken to relax to 50% of the peak twitch tension (HRT) in isolated EDL muscle of the male diabetic group was similar (p=0.677) to the male control group with no significant difference. Maximum fused tetanic tension in isolated EDL muscle of the male diabetic group was less as compared to the male control group which was statistically significant (p<0.001). Maximum fused tetanic tension after the fatigue protocol in isolated EDL muscle of the male diabetic group was significantly lower (p<0.001) as compared to the male control group.

Similarly, tetanic tension after 5 minutes of rest period following the fatigue protocol in isolated EDL muscle of the male diabetic group was less as compared to the male control group which was statistically significant ($p < 0.001$).

DISCUSSION

The present study was designed to evaluate the contractile functions of slow and fast skeletal muscles in streptozotocin (STZ) induced type 1 diabetic male Sprague Dawley rats. At the end of four weeks of study, maximum isometric twitch tension (ITT) in isolated soleus and extensor digitorum longus (EDL) muscles of the male diabetic group was similar to the healthy controls. Skeletal muscle is the predominant tissue for the whole body lipid oxidation, in which up to 90% of energy requirements at rest are derived from fatty acids[14]. T1DM is associated with an increased accumulation of intramyocellular lipids (IMCL), which might serve as an alternate energy source

Table-1: Comparison of contractile properties of isolated soleus muscle between diabetic and control male Sprague Dawley rats.

Contractile properties of soleus muscle	Group I (Male control) n = 15	Group II (Male diabetic) n = 15	*p*-value
Maximum isometric twitch tension (N/g)	1.390 ± 0.021	1.404 ± 0.032	0.153
Time to peak twitch tension (ms)	17.83 ± 1.53	21.85 ± 1.23	< 0.001
Time taken to relax to 50% of the peak twitch tension (ms)	21.44 ± 4.07	33.19 ± 4.14	< 0.001
Maximum fused tetanic tension (N/g)	8.65 ± 0.558	8.95 ± 0.481	0.126
Maximum fused tetanic tension after the fatigue protocol (N/g)	7.30 ± 0.510	5.56 ± 0.619	< 0.001
Tetanic tension after 5 minutes of rest period following the fatigue protocol (N/g)	8.16 ± 0.609	6.49 ± 0.491	< 0.001

All values have been expressed as mean ± SD

Table-2: Comparison of contractile properties of isolated extensor digitorum longus (EDL) muscle between diabetic and control male Sprague Dawley rats.

Contractile properties of EDL muscle	Group I (Male control) n = 15	Group II (Male diabetic) n = 15	p-value
Maximum isometric twitch tension (N/g)	1.385 ± 0.026	1.378 ± 0.025	0.475
Time to peak twitch tension (ms)	7.89 ± 0.114	7.94 ± 0.138	0.342
Time taken to relax to 50% of the peak twitch tension (ms)	6.89 ± 0.089	6.91 ± 0.154	0.678
Maximum fused tetanic tension (N/g)	13.51 ± 0.777	11.46 ± 0.846	< 0.001
Maximum fused tetanic tension after the fatigue protocol (N/g)	5.23 ± 0.084	2.87 ± 0.068	< 0.001
Tetanic tension after 5 minutes of rest period following the fatigue protocol (N/g)	10.52 ± 0.748	7.91 ± 0.786	< 0.001

All values have been expressed as mean ± SD

in the absence of glucose. In addition, in T1DM, intramyocellular lipid accumulation of more than two fold has been observed in the slow soleus muscle as compared to the fast tibialis anterior muscle[15]. Based on the results of present study, it is suggested that the diabetic skeletal muscles derive ATP from intramyocellular lipids to generate normal isometric twitch response.

Time to peak twitch tension (TPT) in isolated soleus muscle of the diabetic group was significantly longer ($p < 0.001$) as compared to the healthy controls. On the contrary, TPT in isolated extensor digitorum longus (EDL) muscle of the male diabetic group was similar to the control group. The characteristic prolongation in time to peak twitch tension in isolated soleus muscle might be explained in part by a change in isomyosin composition of the skeletal muscle in T1DM. This would have resulted in an increase in the number of type I slow-twitch fibers at the expense of type II fast-twitch fibers. Loss of fast isomyosins and appearance of slow isoforms have been demonstrated in a biochemical study on rat's gastrocnemius muscle, 4 weeks after STZ

injection[16]. An impairment of calcium release from the sarcoplasmic reticulum could be another factor for the prolongation in time to peak twitch tension in the diabetic soleus muscle. Previous morphological studies had provided evidence that sarcoplasmic reticulum and T tubules were disrupted in skeletal and cardiac muscles of the diabetic rats[17]. In another study, similar prolongation in time to peak twitch tension in soleus muscle of rats was observed after 14 days of STZ induced T1DM.[18]. Time to peak twitch tension (TPT) was unaffected in extensor digitorum longus muscle in the present study. This reflects that the change in isomyosin form was not of sufficient magnitude to effect the whole muscle measurements, as extensor digitorum longus muscle was still predominantly composed of type IIB or fast-twitch glycolytic fibers.

Time taken to relax to 50% of the peak twitch tension (HRT) in isolated soleus muscle of the male diabetic group was significantly longer ($p < 0.001$) as compared to the control group. On the contrary, HRT in isolated extensor digitorum longus (EDL) muscle of the male diabetic group was similar to the control group.

In present study, the marked slowing in time taken to relax to 50% of the peak twitch tension in isolated soleus muscle is suggestive of impairment in calcium sequestration by sarcoplasmic reticulum. In vitro studies on heart and skeletal muscles of diabetic rats have demonstrated reduced sarcoplasmic reticulum calcium ATPase activity[19]. On the other hand, sarcoplasmic reticulum is much more extensive in the fast muscles which might be the reason that a similar level of damage to the soleus muscle did not have the same effect on extensor digitorum longus muscle, because of its greater functional reserve.

Maximum fused tetanic tension in isolated soleus muscle of the male diabetic group was similar to the control group. On the other hand, maximum fused tetanic tension in isolated extensor digitorum longus (EDL) muscle of the male diabetic group was significantly lower ($p<0.001$) as compared to the control group. This finding highlights the fact that soleus muscle can maintain maximum fused tetanic tension with respect to its muscle mass. This could be associated to the minimal atrophy of type I or slow-twitch oxidative fibers of soleus muscle in T1DM. In a study conducted on 90% pancreatectomized diabetic model of young Sprague Dawley rats, maximum fused tetanic tension was recorded in the gastrocnemius-plantaris-soleus (GPS) muscle complex, after 4 and 8 weeks of diabetes. In that study, maximum fused tetanic tension at 4 weeks was similar between the diabetic (7.95 ± 0.91 N/g) and control (7.86 ± 0.91 N/g) groups[20]. On the other hand, extensor digitorum longus muscle showed a marked decline in force output. This could be due to the preferential atrophy of the predominant fast-twitch glycolytic fibers in extensor digitorum longus muscle which have the greatest tension generating capacity[21]. Hyperglycemia has been associated with profound reduction in protein synthesis and increased protein break down in fast-twitch glycolytic fibers when compared with the slow-twitch fibers[22].

Maximum fused tetanic tension after the fatigue protocol in isolated soleus and extensor digitorum longus (EDL) muscles of the male diabetic group was significantly lower ($p<0.001$) as compared to the control group. The present study reflects that both type I or slow-twitch oxidative fibers and type II or fast-twitch glycolytic fibers exhibit increased fatigability in T1DM. This could be attributed to the common factors affecting type I and type II fibers, such as, reduced ATP production due to the reduced substrate availability, accumulation of metabolic end products that impair contractile events, changes in intracellular and extracellular muscle electrolyte concentration that reduce muscle excitability, and alterations in sarcoplasmic reticulum calcium handling properties[23]. In a study conducted on male Wistar rats, results similar to the present study were observed. In that study, maximum fused tetanic tension in soleus muscle showed 32±3% decline in healthy control rats after 5 minutes of fatigue protocol, whereas, reduction in maximum fused tetanic tension in soleus muscle of the diabetic rats was 52±2% which

was significantly greater (p<0.001). The extensor digitorum longus muscle in diabetic rats also manifested reduction in maximum fused tetanic tension by 72 ± 3% after 5 minutes of fatigue protocol which was significantly greater (p<0.001) than the reduction in tension (63± 3%) observed in healthy control rats[24].

In present study, tetanic tension after 5 minutes of rest period following the fatigue protocol in isolated soleus and extensor digitorum longus (EDL) muscles of the male diabetic group was significantly lower (p<0.001) as compared to the control group. The data of present study highlights that recovery from fatigue is significantly impaired in both type I and type II fibers in T1DM. This could be due to the reduced replenishment of intracellular energy resources (i.e. creatine phosphate, glycogen or ATP), failure of removal of metabolic by products with potential deleterious effects (e.g., H+ and lactate)[25], and changes in muscle ion concentration (e.g., Na^+, K^+, Ca^{+2}, Mg^{+2})[26].

CONCLUSION

It is concluded that streptozotocin induced type 1 diabetes mellitus manifests differential effects on the contractile properties of slow and fast skeletal muscles of male Sprague Dawley rats. In slow muscles, the tetanic tension remains unaffected, while the speed related properties get slowed down. In fast muscles, the tetanic tension is decreased, whereas, the speed related properties remain unaffected. There occurs reduction in resistance to and recovery from fatigue in both slow and fast skeletal muscles.

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