ORIGINAL ARTICLE

SKELETAL MUSCLE CONTRACTILE FUNCTIONS IN STREPTOZOTOCIN INDUCED TYPE 1 DIABETES MELLITUS

Kamil Asghar Imam, Farzana Majeed, Sidra Arshad

Department of Physiology, Army Medical College, National University of Sciences and Technology (NUST), Islamabad, Pakistan

Background: This study was carried out to evaluate the contractile functions of slow and fast skeletal muscles in streptozotocin induced type 1 diabetic female Sprague Dawley rats. **Methods**: Thirty healthy female Sprague Dawley rats were divided into two groups. The rats in group I (female control, n=15) were fed on normal pellet diet and water *ad libitum*. The rats in group II (female diabetic, n=15) were rendered diabetic by single intraperitoneal injection of streptozotocin (STZ) 65 mg/Kg body weight. At the end of four weeks, 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, 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 female diabetic group were significantly longer ($p<0.001$) compared to 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 female diabetic group was significantly lower ($p<0.001$) as compared to the control group. **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 female 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 fibre specific manner. A more rapid down regulation of GLUT4 glucose transporter protein and mRNA expression has been detected in slow-twitch or type I muscle fibres as compared to the fast-twitch or type II fibres.[1] Furthermore, slow-twitch oxidative or type I fibres have shown a greater accumulation of intramyocellular lipids (IMCL).[2]

On the other hand, studies have shown that type IIB or fast-twitch glycolytic fibres undergo the most severe atrophy due to the oxidative stress mediated by hyperglycaemia (e.g., production of advanced glycation end products and reactive oxygen species). The rate of protein breakdown is much greater in type IIB fibres as compared to type I fibres with a parallel decline in protein synthesis.[3]

Much of our current knowledge regarding diabetic myopathy is the result of studies conducted on adult STZ induced diabetic rodents. 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 characterised 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 female Sprague Dawley rats. These muscles were selected to record the contractile functions because they represent two different fibre type populations. The slow soleus muscle is mainly composed of type I or slow-twitch oxidative fibres whereas the fast extensor digitorum longus (EDL) muscle is mainly composed of type II or fast-twitch glycolytic fibres.[7]

MATERIAL AND METHODS

Thirty healthy female Sprague Dawley rats, 80±5 days old and weighing 200–300 grams, were divided into two groups. The rats in group I (female control, n=15) received single intraperitoneal injection of normal saline at the start of study (day 1). The rats in group II (female diabetic, n=15) were rendered diabetic by single intraperitoneal injection of 65 mg/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 $\geq$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 anaesthetised 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% 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, digitise, analyse, 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 evaluated 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 was entered into SPSS-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 female diabetic group (233.27±6.17 mg/dl) was significantly higher ($p<0.001$) compared to the female control group (77.13±2.67 mg/dl). The contractile properties of isolated soleus muscle in the female diabetic rats and healthy controls have been compared in Table-1.

The maximum isometric twitch tension in isolated soleus muscle of the female diabetic group was 1.385±0.029 N/g and in the female control group it was 1.397±0.032 N/g with no significant difference ($p=0.296$). Time to peak twitch tension in isolated soleus muscle of the female diabetic group (21.05±1.17 ms) was significantly longer ($p<0.001$) as compared to the female control group (17±0.998 ms). Time taken to relax to 50% of the peak twitch tension in isolated soleus muscle of the female diabetic group was 30.50±3.76 ms as compared to the female control group (18.98±2.98 ms).

This difference was statistically significant ($p<0.001$). Maximum fused tetanic tension n isolated soleus muscle of the female diabetic group (8.99±0.371 N/g) showed no significant difference ($p=0.084$) when compared with the female control group (8.68±0.556 N/g). Maximum fused tetanic tension after the fatigue protocol in isolated soleus muscle of the female diabetic group (5.92±0.485 N/g) was less as compared to the female control group (7.51±0.433 N/g) which was statistically significant ($p<0.001$). Tetanic tension after reversal from fatigue in isolated soleus muscle of the female diabetic group was 6.84±0.525 N/g and in the female control group it was 8.45±0.517 N/g which was statistically significant difference ($p<0.001$).

Contractile properties of isolated extensor digitorum longus (EDL) muscle in the female diabetic and control groups have been compared in Table-2. Maximum isometric twitch tension (ITT) in isolated EDL muscle of the female diabetic group was 1.394±0.026 N/g and in the female control group it was 1.413±0.028 N/g. Thus, ITT in isolated EDL muscle of the female diabetic and control groups was similar with no significant difference ($p=0.475$).

Table-1: Comparison of contractile properties of isolated soleus muscle between diabetic and control female Sprague Dawley rats at the end of four weeks of study

Contractile properties of soleus muscle	Group I (Female control) n=15	Group II (Female diabetic) n=15	*p*-value
Maximum isometric twitch tension (N/g)	1.397±0.032	1.385±0.029	0.296
Time to peak twitch tension (ms)	17±0.998	21.05±1.17	<0.001
Time taken to relax to 50% of the peak twitch tension (ms)	18.98±2.96	30.50±3.76	<0.001
Maximum fused tetanic tension (N/g)	8.68±0.556	8.99±0.371	0.084
Maximum fused tetanic tension after the fatigue protocol (N/g)	7.51±0.433	5.92±0.485	<0.001
Tetanic tension after 5 minutes of rest period following the fatigue protocol (N/g)	8.45±0.517	6.84±0.525	<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 female Sprague Dawley rats at the end of four weeks of study

Contractile properties of EDL muscle	Group I (Female control) n=15	Group II (Female diabetic) n=15	*p*-value
Maximum isometric twitch tension (N/g)	1.413±0.028	1.394±0.026	0.062
Time to peak twitch tension (ms)	7.93±0.081	7.98±0.109	0.226
Time taken to relax to 50% of the peak twitch tension (ms)	6.91±0.118	6.96±0.142	0.292
Maximum fused tetanic tension (N/g)	14.49±0.916	11.64±0.782	<0.001
Maximum fused tetanic tension after the fatigue protocol (N/g)	5.85±0.092	2.92±0.070	<0.001
Tetanic tension after 5 minutes of rest period following the fatigue protocol (N/g)	11.42±0.931	8.35±0.884	<0.001

All values have been expressed as Mean±SD

Time to peak twitch tension (TPT) in isolated EDL muscle of the female diabetic group was 7.98±0.109 ms and in the female control group it was 7.93±0.081 ms. Thus, TPT in isolated EDL muscle of the female diabetic and control groups was similar with no significant difference (p=0.226).

Time taken to relax to 50% of the peak twitch tension (HRT) in isolated EDL muscle of the female diabetic group was 6.96±0.142 ms and in the female control group it was 6.91±0.118 ms. Thus, HRT in isolated EDL muscle of the diabetic and control female groups was similar with no significant difference (p=0.292).

Maximum fused tetanic tension in isolated EDL muscle of the female diabetic group (11.64±0.782 N/g) was significantly less (p<0.001) as compared to the female control group (14.49±0.916 N/g). Maximum fused tetanic tension after the fatigue protocol in isolated EDL muscle of the female diabetic group (2.92±0.070 N/g) was lower as compared to the female control group (5.85±0.092 N/g). This difference was statistically significant (p<0.001).

Tetanic tension after reversal from fatigue in isolated EDL muscle of the female diabetic group (8.35±0.884 N/g) was significantly less (p<0.001) as compared to the female control group (11.42±0.931 N/g).

DISCUSSION

The present study was designed to measure the contractile functions of slow and fast skeletal muscles in streptozotocin (STZ) induced type 1 diabetic female 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 female diabetic group was similar to the control group. Skeletal muscle is the predominant tissue for the whole body lipid oxidation, in which up to 90% of energy requirements at rest are obtained from fatty acids.[14] T1DM is associated with an increased accumulation of intramyocellular lipids (IMCL), which might serve as an alternate energy source in the absence of glucose. In addition, in T1DM, intramyocellular lipid accumulation of more than two folds 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 produce 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 healthy controls. On the contrary, TPT in isolated extensor digitorum longus (EDL) muscle of the female diabetic group was similar to the control group. The characteristic slowing 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 fibres at the expense of type II fast-twitch fibres. 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 have shown that sarcoplasmic reticulum and T tubules

are disrupted in skeletal and cardiac muscles of the diabetic rats.[17] 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 fibres.

Time taken to relax to 50% of the peak twitch tension (HRT) in isolated soleus muscle of the female 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 female 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.[18] 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 female 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 female diabetic group was significantly lower (p<0.001) as compared to the control group. Thus, the present study highlights 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 fibres of soleus muscle in T1DM. On the other hand, extensor digitorum longus muscle showed a profound decline in force output. This could be due to the preferential atrophy of the predominant fast-twitch glycolytic fibres in extensor digitorum longus muscle which have the highest tension generating capacity.[19] Hyperglycaemia has been associated with profound reduction in protein synthesis and increased protein degradation in fast-twitch glycolytic fibres when compared with the slow-twitch fibres.[20]

Maximum fused tetanic tension after the fatigue protocol in isolated soleus and extensor digitorum longus (EDL) muscles of the female diabetic group was significantly lower (p<0.001) compared to the control group. The present study reflects that both type I or slow-twitch oxidative fibres and type II or fast-twitch glycolytic fibres exhibit increased fatigability in T1DM. This could be attributed to the common factors affecting type I and type II fibres, 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.[21]

In present study, tetanic tension after 5 minutes of rest period following the fatigue protocol in isolated soleus and EDL muscles of the female 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 fibres 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)[22], and changes in muscle ion concentration (e.g., Na^+, K^+, Ca^{+2}, Mg^{+2}).[23]

CONCLUSION

Streptozotocin induced type 1 diabetes mellitus exerts differential effects on the contractile properties of slow and fast skeletal muscles of female 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 is reduction in resistance to and recovery from fatigue in both slow and fast skeletal muscles.

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Address for Correspondence:

Dr. Kamil Asghar Imam, Department of Physiology, Army Medical College, National University of Sciences and Technology, Islamabad. Pakistan. **Tel:** +92-51-5156789; +92-344-9144488
Email: kkamzz@hotmail.com