COMPARISON OF CONTRACTILE PROPERTIES BETWEEN SLOW AND FAST SKELETAL MUSCLES OF FEMALE DIABETIC SPRAGUE-DAWLEY RATS

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ABSTRACT

Objective: To compare isometric contraction, force-frequency relationship and muscle fatigue between slow and fast muscles of female type 2 diabetes mellitus Sprague-Dawley rats.

Study Design: Experimental study.

Place and Duration of Study: Physiology Department, Army Medical College, Rawalpindi and National Institute of Health, Islamabad from Jan to Dec 2015.

Material and Methods: Twenty healthy female Sprague Dawley rats were divided into 2 groups with 10 rats in each group. Group-I (control) was fed with normal diet and group-II (diabetic) was given high fat diet. Group-II was given intraperitoneal streptozotocin (STZ) (35mg/kg body weight) on 15th day. Body weight, blood glucose and TG: HDL ratio were estimated on 21st day to confirm type II diabetes mellitus (T2DM) induction. Soleus and extensor digitorum longus (EDL) muscles were removed intact and fixed in organ bath system containing Krebs Ringer buffer solution and connected to data acquisition unit (iWorx) to study their contractile parameters.

Results: Isometric twitch tensions of slow (soleus) and fast (EDL) muscles were similar in diabetic and control rats. Contraction and half relaxation times were slower in diabetic soleus muscles in comparison to control muscles. Diabetic soleus and EDL muscles displayed significantly ($p<0.05$) increased fatigability.

Conclusion: In STZ induced type II diabetic slow muscles, the tetanic tension remains unaffected while contraction and half relaxation times were longer. In fast muscles, tetanic tension and the speed related properties remain unaffected. There was reduction in resistance to and recovery from fatigue in both slow and fast skeletal muscles.

Keywords: Fast twitch muscle, Female rats, Slow twitch muscle, Type 2 diabetes mellitus.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a global epidemic with a prevalence rising swiftly on an annual basis. It is a metabolic disorder that also affects peripheral tissues including skeletal muscle. There are two classes of skeletal muscle fibers; type 1 (slow/oxidative) and type 2 (fast/glycolytic). The type 2 muscle fibers have three subtypes; type 2A, 2X and 2B[1]. Slow soleus (~50:50 of type 1 and 2A fibers) and fast extensor digitorum longus (EDL) (50:50 of 2A and 2B fibers) muscles of rats represent two distinct fiber type populations[2]. Female muscles are generally fatigue resistant and recover faster. Type 2 diabetic muscles exhibit impaired mitochondrial function due to reduced oxidative enzyme activity. These muscles become weak and develop a tendency to fatigue early due to reduced energy reserves and inability to restore these reserves efficiently. Chronic T2DM leads to muscle weakness and decline in muscle functions[3]. Early muscle fatigue can affect the quality of life as it reinforces the tendency to physical inactivity that is commonly observed in the diabetic patients. Physical inactivity has been considered as an important risk factor for the increased incidence of diabetes. The decline in muscle functions due to T2DM has not been studied based on gender to our knowledge. The present study was designed to compare the contractile properties of soleus and extensor digitorum longus (EDL) skeletal muscles in STZ

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Received: 10 Dec 2015; revised received:23 May 2016; accepted: 22 Jun 2016

induced female type 2 diabetic Sprague Dawley rats.

MATERIAL AND METHODS

The study was conducted in the research laboratory of Physiology department at Army Medical College in collaboration with National Institute of Health (NIH), Islamabad after formal approval from Ethical Review Committee. Twenty healthy female Sprague Dawley rats about 90 ± 5 days old and weighing 250 ± 50 gm were included in the study via non-probability convenience sampling and divided into control and diabetic groups with 10 rats each. The rats with diabetes (blood glucose measurement) or having any muscular disease (measured by creatine phosphokinase or CPK levels) were excluded. They were kept in separate 2×3 feet steel cages and light and dark cycles of 12:12 hours plus an optimal temperature of 20-22^{0}C were maintained. The control rats were fed with normal pellet diet (NPD) and diabetic group was given high fat diet (HFD)[4]. On 15th day of the study, the diabetic group was administered 35mg/kg body weight streptozotocin (STZ) intraperitoneally in the lower right quadrant of the abdomen to induce T2DM. On the 21st day, after an overnight fast the blood glucose (>16.65 mmol/l or 301 mg/dl) and triglyceride and high density lipoprotein ratio (TG:HDL) >1.8 were observed which confirmed the development of T2DM in diabetic group (table-I)[4]. Afterwards, rats were anaesthetized by ether inhalation[4] and their slow and fast muscles (that is soleus and EDL) were removed intact. Their distal tendons were tied by non-absorbable surgical silk and fixed with a support while proximal tendons were tied to the force transducer (FT-100) connected to iWorx advanced animal/human physiology data acquisition unit (AHK/214)[5]. Whole muscle was mounted in a 25 ml organ bath system containing Krebs-Ringer buffer solution. It was continuously bubbled with a mixture of 95% O2 (oxygen) and 5% CO2 (carbon dioxide). Temperature of 30˚C was maintained by a thermostat[6].

Table-I: Comparison of body weight, blood glucose levels and TG:HDL ratio between control and diabetic Sprague-Dawley rats.

Variables	Days	Control Group	Diabetic Group	*p* value
Body weight (gm)	Day 1	277.80 ± 17.74	261.80 ± 8.26	0.023
	Day 21	293.70 ± 17.93	361.90 ± 25.85	0.001*
Blood sugar (mg/dl)	Day 1	125.60 ± 14.19	120.60 ± 12.74	0.418
	Day 21	132.00 ± 15.25	362.8.0 ± 28.28	0.001*
TG:HDL	Day 1	0.95 ±0.23	0.93 ±0.18	0.840
	Day 21	1.08 ± 0.54	3.26 ± 1.44	0.001*

All values have been expressed as mean ± SD.
*p-value <0.05

Table-II:Comparison of contractile properties of isolated soleus (slow) muscles between diabetic and control groups of Sprague Dawley rats.

Contractile properties of soleus muscle	Control group n=10	Diabetic group n=10	*p* value
Maximum isometric twitch tension (N/g)	1.75 ± 0.72	1.64 ± 0.79	0.736
Time to peak twitch tension (ms)	2.83 ± 0.49	3.73 ± 0.85	0.009*
Half relaxation time (ms)	1.75± 0.66	2.42 ± 0.62	0.032*
Maximum fused tetanic tension (N/g)	0.16 ± 0.27	0.03 ± 0.03	0.135
Maximum fused tetanic tension after fatigue protocol (N/g)	0.09 ± 0.09	0.01 ± 0.01	0.023*
Tetanic tension after 5 minutes of rest period following fatigue protocol (N/g)	0.02 ± 0.01	0.004 ± 0.01	0.001*

All values have been expressed as mean ± SD.
*p-value <0.05

Muscles were stimulated using single (1Hz) twitch stimulations with 1 minute rest periods to record isometric twitches, time to peak twitch tension (TPTT) and time taken to relax to 50% of its peak twitch tension or half relaxation time (HRT)[7]. Force-frequency relationship was determined by recording the tension produced after stimulating the muscle at increasing frequencies (10 to 110 Hz) for 1 second followed by rest of 3 minutes in between. The maximum tetanic force was calculated. Muscle fatigue was induced and recorded after stimulating the muscle with a one second optimum tetanic stimulation every 5 seconds for 5 minutes. A measure of recovery from fatigue was made by recording the tetanic tension after 5 minutes rest period following the fatigue protocol. All muscle tensions were expressed as Newton/gram (N/g) wet muscle mass[6]. Statistical analysis of data was done by using SPSS version 21. Independent samples t test was applied and a *p*-value <0.05 was considered significant.

RESULTS

The body weight, plasma glucose and CPK of all rats were within normal range initially. On 21st day T2DM induction was confirmed based on increased blood glucose levels and TG:HDL ratio (table-I). Body weight of diabetic group was increased significantly (*p*=0.001).

There was no change in isometric twitch tension in both soleus i.e. slow (table-II) and EDL i.e. fast (table-III) muscles of diabetic group as compared to their control counterparts. The TPTT and HRT were significantly prolonged in isolated soleus but not in EDL muscles of diabetic group. The maximum fused tetanic tension (MFTT) was significantly reduced (*p*=0.044) in isolated EDL but not in soleus muscle (*p*=0.135) of diabetic group. The MFTT after fatigue protocol and tetanic tension after 5 minutes of rest period following fatigue protocol were significantly reduced in both isolated soleus and EDL muscles of diabetic group (tables II and III).

DISCUSSION

The animal model comprising of feeding high fat diet (HFD) and low dose STZ administration was considered feasible due to its easy access and cost effectiveness[8]. Srinavasan also recommended a similar animal model for T2DM induction[4].

Significant (*p*= 0.001) increase in body weight of diabetic rats at the end of the study occurred due to the accumulation of intra-myocellular lipid droplets which contributed in the development of insulin resistance in response to HFD intake[9]. Comparable changes in weight gain had been observed in a study conducted on diabetic male Sprague Dawley rats after feeding HFD[10]. The diabetic group in our study developed frank hyperglycemia. High fat diet was a contributor and STZ reduced the insulin secretion which caused the development of frank hyperglycemia as it destroyed the β cells of the pancreas. Streptozotocin induced T2DM in

Table-III: Comparison of contractile properties of isolated EDL (fast) muscles of diabetic and control groups.

Contractile properties of EDL muscle	Control group n=10	Diabetic group n=10	*p* value
Maximum isometric twitch tension (N/g)	1.89 ± 0.82	2.16 ± 0.89	0.492
Time to peak twitch tension (ms)	1.55 ± 0.52	1.91 ± 0.10	0.058
Half relaxation time (ms)	1.12 ± 0.50	1.27 ± 0.29	0.429
Maximum fused tetanic tension (N/g)	0.33 ± 0.33	0.08 ± 0.05	0.044*
Maximum fused tetanic tension after fatigue protocol (N/g)	0.11 ± 0.09	0.01 ± 0.01	0.016*
Tetanic tension after 5 minutes of rest period following fatigue protocol (N/g)	0.02 ± 0.01	0.004 ± 0.01	0.019*

All values have been expressed as mean ± SD
**p*-value <0.05

Wistar Furth rats produced frank hyperglycemia in female rats (24.6 mmol/l or 443.24 m/dl)[11].The higher levels attained in this study could be attributed to the higher dose of STZ (55mg/kg body weight) used. In a study conducted on diabetic female adult albino Wistar rats, the mean TG:HDL ratio was 2.84[12] which is relatively lesser compared to our study (3.26 ± 1.44) probably due to the difference in species of rats or the dose of STZ (40mg/kg) administered.

The MITT of isolated soleus and EDL muscles was similar between diabetic and control groups as it depends on the number of cross linkages between the myosin heads and actin filaments at a given time. These are dependent on ATP and Ca+2 in the sarcoplasm which are sufficient in diabetic muscle for a single twitch. A study on diabetic Wistar rats weighing 200 to 220 gm displayed minimal effect on isometric twitch tension in diabetic rats[13].

The TPTT was found significantly longer in isolated soleus muscle but not in EDL muscle of diabetic group. It depends on the efficient release of calcium from sarcoplasmic reticulum which was not affected in fast muscles. The prolonged HRT in soleus muscle is due to increased type 1 fibers leading to a relative reduction in fast fibers especially 2A fibers causing the delay in calcium release[14]. Similar significant (p<0.05) increase in contraction time was observed in STZ induced T2DM rats[10]. An insignificant difference in TPTT of fast muscles was reported in a study conducted on T2DM Sprague Dawley rats[15].

The HRT of diabetic soleus muscle (table-II) was significantly prolonged while that of EDL was similar to the healthy controls (table-III). A less dense SR in slow muscles caused slower release and uptake of calcium and slower kinetics of contraction[2]. Similar results were obtained in a study conducted on diabetic Wistar rats whose soleus muscle had a prolonged HRT compared to control group[13]. Abundant calcium stores in diabetic fast muscles for the single twitch did not alter their HRT. Diabetic rats after T2DM induction did not show significant increase in HRT of diabetic EDL muscles[5].

The MFTT was comparable in isolated diabetic soleus muscle but significantly reduced in diabetic EDL muscles. It depends on strongly bound cross bridges with an increased calcium concentration and ATP to generate greater force. The intra-myocellular lipid accumulation in type 1 fibers provides an added source of ATP. Similar observations were recorded in a study conducted on diabetic rats in which MFTT was comparable in diabetic and control groups[16]. Subject to 12 hours fast, EDL muscles in this study had a reduced muscle glycogen content which was not compensated in the insulin resistant state causing a drop in tetanic tension[9]. Study conducted on T2DM rats revealed similar significant (p=0.029) lowering of MFTT in diabetic EDL muscles[10].

Data suggest significantly increased fatigability in both soleus and EDL muscles of diabetic rats of our study when compared with controls. The diabetic EDL muscles in our study were more fatigued than soleus. Sustained contractions cause reduction in fuel availability in diabetic muscles faster than the controls which impaired the functioning of contractile apparatus leading to their early fatigue and recovery from fatigue was delayed due to their inability to efficiently replenish energy resources[17]. A study conducted on diabetic rats showed similar lowering of maximum fused tetanic tension after fatigue protocol in rats of chronic diabetes of 2 months duration[13]. Another study on T2DM Sprague Dawley rats confirmed the increased fatigability in diabetic slow and fast muscles[18].

These findings and further studies based on enzymatic analysis of the skeletal muscle fibers can be utilized for improving the management of type 2 diabetic patients and planning exercise regimens for improving muscle function.

CONCLUSION

In STZ induced type 2 diabetic slow muscles, the tetanic tension remains unaffected while contraction and half relaxation times are longer. In fast muscles, the tetanic tension and the speed related properties remain unaffected. There is reduction in resistance to and recovery from fatigue in both slow and fast skeletal muscles.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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