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Original Article

Skeletal Muscle Fatigue in Slow and Fast Muscles of Type 2 Diabetic Sprague Dawley Rats

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Abstract

Background: To evaluate the effects of type 2 diabetes mellitus on fatigability of soleus (slow) and extensor digitorium longus (EDL) (fast) muscles of female Sprague Dawley rats.

Methods:Twenty healthy female Sprague Dawley rats were divided into 2 groups with 10 rats each. Group I (control) was fed with normal diet and group II (diabetic) was given high fat diet. Group II was given intra-peritoneal 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 2 diabetes mellitus (T2DM) induction. Soleus and extensor digitorium 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 and fatigability parameters.

Results: Soleus and extensor digotorium longus muscles of female diabetic rats displayed significantly ($p<0.05$) increased fatigability.

Conclusions:Fatigability of slow (soleus) and fast (EDL) muscles increases in T2DM.There occurs reduction in resistance to and recovery from fatigue in both slow and fast skeletal muscles of type 2 diabetic female Sprague Dawley rats.

Keywords: Fast twitch muscle,Slow twitch muscle, Muscle fatigue, Type 2 diabetes mellitus

Introduction

Deleterious effects of type 2 diabetes mellitus especially on the musculoskeletal system have been a global concern for past few decades. Diabetic muscles are believed to fatigue earlier than usual and lose their contractile power. Female muscles are considered more fatigue resistant and to recover faster. Type 2 diabetes mellitus (T2DM) is an adult onset diabetes that occurs in individuals with insulin resistance and relative insulin deficiency.[1] Skeletal muscles of rats exhibit insulin dependent glucose uptake via glucose transporter (GLUT 4) located in the plasma membrane.[2] Type 1 and 2 skeletal muscle fibers are uniquely distributed among slow (soleus) and fast (EDL) muscles of rats such that the EDL muscle of rats possess ~50:50 blend of 2A and 2B fast fibers and the soleus comprises 50:50 of 1 and 2A fibers.[3] Skeletal muscle insulin resistance (inefficient utilization of insulin for glucose homeostasis) is considered the primary defect for T2DM evolution. T2DM brings about alterations in the machinery and working of skeletal muscles to produce multi-organ effects.[4]

Impaired mitochondrial function due to reduced oxidative enzyme activity is exhibited by type 2 diabetic muscles. These muscles have reduced energy reserves and are unable to efficiently restore these reserves and therefore develop tendency to fatigue early. More pronounced down regulation of GLUT4 glucose transporter protein has been evidenced in slow-twitch type1muscle fibers in comparison to the fast-twitch type 2 fibers.Additionally, slow-twitch oxidative fibers accumulate more intramyocellular lipids.Conversely, type 2B or fast-twitch glycolytic fibers have shown greater atrophic changes due to hyperglycemia induced oxidative stress (e.g., production of advanced glycation end products and reactive oxygen species). Type 2B fibers also exhibit greater rate of protein breakdown and decreased protein synthesis as compared to type 1 fibers.[5]

A significant association exists between physical inactivity and high fatigability in type 2 diabetic females. Female muscles have been found to be more resistant to fatigue and they recover from fatigue faster due to their sex hormonal status.[6]

Material and Methods

The project was formally approved by Ethical Review Committee after which it was piloted at research laboratory of Physiology department, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad. Twenty healthy female Sprague Dawley rats divided into control and diabetic groups with 10 rats each were included in the

study via non-probability convenience sampling.All rats were 90 ± 5 days old and weighed 250 ± 50 gm. Diabetic rats (diagnosed by blood glucose measurement) or those with muscular disease (measured by creatine phosphokinase (CPK) level 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 20-22C were maintained. Normal pellet diet (NPD) was fed to the control rats and diabetic rats were given high fat diet (HFD)[7].T2DM was induced in the diabetic group by intra-peritonealstreptozotocin (STZ)administration(35mg/kg body weight)on the 15th day of study. After an overnight faston the 21st day, blood glucose (>16.65mmol/l or 301mg/dl) and triglyceride to high density lipoprotein ratio (TG:HDL) >1.8 were observed confirming the development of T2DM in diabetic group.[7] The slow and fast muscles (i.e. soleus and EDL)of these rats were removed intact after anaesthetization with ether inhalation[7].The distal tendons of the muscles were tied by non-absorbable surgical silkand 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).[8] Whole muscle was mounted in a 25-ml organ bath systemcontaining Krebs-Ringer buffer solution. It was continuously bubbled with a mixture of 95% O_2 (oxygen) and 5% CO_2 (carbon dioxide). Temperature of 30˚C was maintained by a thermostat.[9]The force-frequency relationship was estimated by recording the tension produced after stimulating the muscle at increasing frequencies (10 to 110 Hz) for 1 second followed byrest of 3 minutes in between. The maximum tetanic force was calculated. The induction and recording of muscle fatigue was done after stimulating the muscle with a 1 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[9].SPSS version 21 was used to statistically analyze the data. Independent samples t-test was applied and p-value<0.05 was considered significant.

Results

At start of study, the body weight, plasma glucose and CPK of all rats were normal. On 21st day T2DM induction was confirmed based on increased blood glucose levels and TG:HDLratio (Table 1). Mean body weight of diabetic group was also increased significantly (p<0.001).Rats when fed with HFD, and administered low dose of STZ (35mg/kg body weight) developed frank hyperglycemia, weight gain and increased TG:HDL ratio which manifested as development of insulin resistance.

Table 1: Comparison of body weight, blood glucose levels and TG:HDL ratio between control and diabetic female 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 2: Comparison of parameters of muscle fatigue of isolated soleus muscles between diabetic and control groups of female Sprague Dawley rats

Properties of muscle fatigue in soleus muscle	Control Group n = 10	Diabetic Group n = 10	p-value
Maximum fused tetanic tension (N/g)	0.16 ± 0.27	0.03 ± 0.03	0.135
Maximum fused tetanic tension after the fatigue protocol (N/g)	0.09 ± 0.09	0.01 ± 0.01	0.023
Tetanic tension after 5 minutes of rest period following the 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

Table 3: Comparison of parameters of muscle fatigue of isolated EDL muscles of female diabetic and control groups

Properties of muscle fatigue in EDL muscle	Control Group n = 10	Diabetic Group n = 10	p-value
Maximum fused tetanic tension (N/g)	0.33± 0.33	0.08 ± 0.05	0.044
Maximum fused tetanic tension after the fatigue protocol (N/g)	0.11± 0.09	0.01 ±	0.016
Tetanic tension after 5 minutes of rest period following the 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

Blood glucose level ≥ 300mg/dl and TG:HDL ratio of >1.8 were taken as cut off values for the development of T2DM.There was no significant (p=0.135) change in MFTT of isolated soleus muscle of diabetic rats of our study when compared with controls.Maximum fused tetanic tension after fatigue protocol was significantly (p=0.023) reduced in isolated soleus muscle of diabetic rats (Table 2). The tetanic tension after 5 minutes rest of fatigue protocol was significantly (p=0.001) reduced in isolated soleus muscle of diabetic rats.The MFTT in EDL muscles of diabetic rats was significantly (p=0.016) reduced in comparison to control counterparts.The MFTT after fatigue protocol was significantly (p=0.016) reduced in diabetic group.The tetanic tension after 5 minutes rest of fatigue protocol in isolated EDL muscle was significantly (p=0.019) different than the controls (Table 3).

Discussion

The animal model applied in this study comprising of feeding high fat diet (HFD) with low dose STZ administration was considered appropriate due to its cost effectiveness and easy access[10]. This model has been adopted by Srinavasan and his associates.[7] Sprague Dawley rats were the choice for this animal model as they are easily available inbred rodents and develop a disease state that closely mimicks the human metabolic syndrome.[7] Some researchers have used genetic models such as Zucker diabetic fatty (ZDF) rat and db/db mouse in which similar disease state as T2DM was established but these strains were not easily available and were expensive.[11] Streptozotocin (STZ) is taken up by the β cells of pancreas via GLUT 2 (glucose transporter) where it produces cytotoxic effects. These effectsof STZ on β cells are believed to be due to alkylation of DNA and formation of reactive oxygen species (ROS).[12]

Rats when fed with HFD and administered low dose of STZ (35mg/kg body weight) developed frank hyperglycemia, weight gain and increased TG:HDL ratio which manifested as development of insulin resistance. These levels were consistent with animal model devised by Srinavasan and associates.[7] Raised blood glucose in diabetic rats (362.8 ± 28.28 mg/dl)of our study was observed in response to HFD and STZ administration which reduced insulin secretion and led to development of frank hyperglycemia as it destroyed β cells of pancreas.Comparable reults were also seen in a study on STZ (35mg/kg) treated Sprague Dawley rats whose glucose levels reached 19.50 ± 0.76 mmol/l (351.35mg/dl).[13] The diabetic rats in our study exhibited prominent weight gain probably due to accumulation of intramyocellular lipid droplets which contributed in the development of insulin resistance in response to HFD intake.The diabetic rats showed significantly (p<0.001) increased body weight as compared to controls.Comparable increase in body weight has been observed in a local study conducted on Sprague Dawley rats treated with STZ (35mg/kg body weight) and HFD with an initial weight of 250 ± 10gm.[14] The TG:HDL ratio >1.8 was taken as confirmatory for insulin resistance.The diabetic group developed a ratio of 3.26 ± 1.44.Comparable results were observed in a local studty on Sprague Dawley rats treated with STZ (35mg/kg) and HFD for 2 weeks.[15] A study conducted on female adult albino Wistar rats treated with STZ showed TG:HDL ratio of 2.84 to suggest development of insulin resistance. The realtively lesser value compared to our study (i.e. 3.26) might be due to the difference in species of rats, the dose of STZ (40mg/kg) administered and the initial weight of rats (180-200gm) at the time of induction into the study.[16]

Soleus and EDL muscles were dissected intact from each ratafter confirming T2DM and their fatigue parameterswere studied. Soleus muscle is blend of predominantly oxidative type 1 fibers and few fast twitch oxidative glycolytic type 2A fibers while EDL muscles mianly possess fast fibers (2A and 2B) which

are glycogen rich.[3] On 21st day of the study, the weight of isolated soleus and EDL muscles was significantly ($p<0.001$) reduced in diabetic rats as compared to the healthy controls. Similar reduction in weight of soleus and EDL muscles has been documented in STZ treated Sprague Dawley rats.[17] Muscles undergo deleterious effects including atrophy in response to ROS generated via oxidative stress in T2DM.[12]

In present study muscles were stimulated at different frequencies by using a force transducer to record the parameters of fatigue.The maximum fused tetanic tension (MFTT) is the outcome of strongly bound cross bridges acting in parallel to generate greater force. There was no significant ($p=0.135$) change in MFTT of isolated soleus muscle of diabetic rats of our study when compared with controls suggesting that slow diabetic muscles can maintain MFTT. This can be due to type 1 fibers undergoing lesser atrophy than fast fibers as they are dependent on oxidative pathways and have sufficient ATP to maintain the MFTT.A study on STZ induced diabetic Wistar rats confirmed a non signficant ($p>0.05$) difference in MFTT of soleus muscle between diabetic and control groups.[18]

Maximum fused tetanic tension after fatigue protocol was significantly ($p=0.023$) reduced in isolated soleus muscle of diabetic rats showing increased fatigability in slow soleus muscles of diabetic rats of our study. Sustained contractions cause reduction in fuel availability (ATP) in diabetic muscles leading to their early fatigue. Accumulation of metabolic end products impair the functioning of contractile and calcium handling apparatus.[19] Comparable results were seen in STZ induced rats of chronic diabetes which showed increased fatigabilityin slow soleus muscles.[20] The tetanic tension after 5 minutes rest of fatigue protocol was significantly ($p=0.001$) reduced in isolated soleus muscle of diabetic rats suggesting impairment of recovery from fatigue in slow muscles.This might be due to the inability of diabetic muscle to efficiently replenish intracellular energy resources (creatine phosphate, glycogen or ATP).Closely mimicking results were seen in STZ induced diabetic rats revealing significantly increased fatigability and reduced recovery from fatigue.[21]

The MFTT in EDL muscles of diabetic rats was significantly ($p=0.016$) reduced in comaprison to control counterparts indicating increased fatigability in T2DM. The muscle glycogen provides ATP during sustainsed teatnic contractionswhich is reduced in insulin resistant states.[22] Astudy conducted on STZ induced Sprague Dawley rats revealed similar significant ($p=0.029$) lowering of MFTT in diabetic rats.[14]The MFTT after fatigue protocol was significantly ($p=0.016$) reduced in diabetic group.Diabetic muscles lose their ATP faster than the control muscles after repeated stimulations at tetanic frequencies. A study conducted on STZ induced Sprague Dawley rats confirmed the significant ($p=0.002$) reduction in MFTT after fatigue protocol in diabetic EDL muscle.[15] The tetanic tension after 5 minutes rest of fatigue protocol in isolated EDL musclewas significantly ($p=0.019$) different than the controls. This suggests that diabetic muscles could not recover enough fuel stores to generate adequate contractile force but controls restored their fuel efficiently. Comparable significance ($p=0.03$) was seen in STZ induced diabetic Sprague Dawley rats when their recovery from fatigue was measured in EDL muscles.[8]

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Conclusions

1.There is an increased fatigability in rats after extracting T2DM. Female muscles appeared as more fatigue resistant compared to males.

2.Fatigability of slow (soleus) and fast (EDL) muscles inceases in T2DM. There occurs a reduction in resistance to and recovery from fatigue in both slow and fast skeletal muscles.

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