ORIGINAL ARTICLE

PROPERTIES OF FATIGUE BETWEEN SLOW AND FAST MUSCLES OF MALE AND FEMALE DIABETIC RATS

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Background: The metabolic disorder of Type 2 Diabetes Mellitus (T2DM) has deleterious effects on skeletal muscle contractile functions. This study aimed to compare parameters of fatigue between slow and fast skeletal muscles of male and female type 2 diabetic Sprague-Dawley rats. **Methodology:** This was a quasi experimental study conducted at Army Medical College Rawalpindi and National Institute of Health, Islamabad. Forty Sprague Dawley rats in good health were split into groups I (Control) and II (Diabetics) of 20 rats each. Each group had 10 male and 10 female rats. Diabetic groups were given intra-peritoneal streptozotocin (STZ), 35 mg/Kg body weight on 15th day. Control groups were fed a normal diet and diabetic rats were given diet with high fat. On 21st day body weight, blood glucose and triglyceride/high density lipoprotein ratio were estimated to confirm extraction of type 2 diabetes mellitus. Intact Soleus and Extensor Digitorium Longus muscles were removed and fixed in organ bath system having Krebs-Ringer buffer solution and connected to data acquisition unit (iWorx®) to study their fatigability parameters. Independent samples *t*-test was used for statistical analysis. **Results:** Slow and fast skeletal muscles in diabetic rats showed reduction in resistance to and recovery from fatigue. Fatigue parameters between male and female diabetic rats revealed no significant differences. **Conclusion:** Fatigue parameters do not differ between male and female diabetic rats.

Keywords: Type 2 Diabetes mellitus, fast twitch muscle, muscle fibre, fatigue, slow twitch muscle

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INTRODUCTION

The metabolic disorder of Type 2 Diabetes Mellitus (T2DM) has deleterious effects on skeletal muscle contractile functions. The International Diabetes Federation (IDF) has calculated that 11.5 million people in Pakistan will be diabetic by the year 2025.[1] The T2DM pooled prevalence in Pakistan is 13.7% according to a meta-analysis conducted in 2020.[2] Diabetic muscles fatigue early and lose their physiological strength. Male muscles are believed to be comparatively less fatigue resistant than female muscles and adult onset T2DM individuals have a relative insulin deficiency with insulin resistance.[3] In T2DM, gender is a significant determinant of skeletal muscle contractile force and muscle mass based on their skeletal muscle fibres composition.[4] Glucose uptake in rat skeletal muscles is dependent on insulin via glucose transporter (GLUT 4).[5] Fast extensor digitorium longus (EDL) muscles of rats comprises of ~50:50 blend of 2A and 2B fast skeletal muscle fibres while slow (soleus) muscles possess 50:50 of 1 and 2A fibers.[6]

Primary defect in development of T2DM is insulin resistance in skeletal muscles which demonstrates disturbed mitochondrial function in response to decreased oxidative enzyme activity. Diabetic muscles have fewer energy reserves. Limited efficiency to restore these reserves leads to early fatigue.[7] Type 1 slow-twitch muscle fibres exhibit more down regulation of GLUT 4 in comparison to type 2 fast-twitch fibers.[7] Intramyocellular lipid accumulation is more pronounced in oxidative slow twitch fibres while glycolytic fast twitch (2B) fibres exhibit more atrophic changes in response to oxidative stress by hyperglycaemia. Rate of protein breakdown is higher than its synthesis among type 2B fast fibers.[7] The levels of oxidative enzymes is reduced while glycolytic enzymes were found to be raised in obese and diabetics.[8]

Increased fatigability and physical inactivity are significantly associated with type 2 diabetes.[8] The current study was conducted to compare the parameters of fatigue between soleus and EDL muscles in streptozotocin (STZ) induced T2DM male and female Sprague Dawley rats in order to identify any gender specific differences.

MATERIAL AND METHODS

This quasi experimental study after formal approval by Ethical Review Committee was carried out in one year at research laboratory of Physiology Department, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad. A total of 40 healthy male and female Sprague Dawley rats were split into diabetic and control groups via non-probability convenience sampling with10 rats in each group. Group Ia (male control), Group Ib (Female Control), Group IIa (male diabetic) and Group IIb (female diabetic). All included rats weighed 250±50 gm while their age was 90±5 days. The rats excluded were either diabetic

(diagnosed by blood glucose measurement) or had muscular disease (measured by creatine phosphokinase level).

All rats included in the study were housed in 2×3 feet steel cages and light and dark cycles of 12:12 hours was ensured. Temperature was kept optimal at 20–22 °C. Control and diabetic rats were fed normal pellet diet (NPD) and high fat diet (HFD) respectively[7].

An intra-peritoneal streptozotocin 35 mg/kg body weight was administered on the 15th day of study to the diabetic groups for induction of T2DM. On the 21st day after fasting overnight, estimation of blood glucose (>16.65 mmol/l or 301 mg/dl) and triglyceride to high density lipoprotein ratio (TG:HDL) >1.8 confirmed T2DM induction in these groups.[7] After anaesthetization of rats with ether inhalation their slow and fast muscles (soleus and EDL) were dissected out intact.[5] A non-absorbable surgical silk was used to tie the distal tendons of the muscles which were then fixed with a support. The proximal tendons were tied to the force transducer (FT-100) that was connected to iWorx® advanced animal/human physiology data acquisition unit (AHK/214).[7,9] The whole muscle was mounted on a 25 ml organ bath system which was filled with Krebs-Ringer buffer solution. A mixture of 5% CO_2 and 95% O_2 was continuously bubbled in it. Temperature was kept optimal by a thermostat at 30 °C.[7,9]

The first parameter of fatigue recorded was maximum fused tetanic tension (MFTT) by stimulating the muscle every 5 seconds with a 1 second optimal tetanic stimulation for 5 minutes. Recovery from muscle fatigue was ensured by 5 minutes rest after the protocol of fatigue. After this rest the tetanic tension was noted. All muscle tension parameters were designated as Newton/gram (N/g) wet muscle mass.[7,9]

SPSS-21 was used to analyze the data statistically. Independent samples *t*-test was employed for generation of results and $p<0.05$ was deemed significant.

RESULTS

Body weight, blood glucose and TG:HDL were measured both at the start and end of study and these parameters at day 21 confirmed the extraction of T2DM in both diabetic groups (Table-1). Parameters of fatigue were compared among male and female diabetic groups for both slow and fast muscles which did not show any significant difference ($p>0.05$) as shown in Table-2 and 3 respectively.

Table-1: Body weight, blood glucose levels and TG:HDL ratio between control and diabetic groups

Variables	Days	Group Ia Male Control	Group IIa Male Diabetic	*p*	Group Ib Female Control	Group IIb Female Diabetic	*p*
Body weight (gm)	Day 1	270.80±19.01	262.80±11.47	0.269	277.80±17.74	261.80±8.26	0.023
	Day 21	289.20±17.44	336.60±22.81	<0.001	293.70±17.93	361.90±25.85	<0.001
Blood sugar (mg/dl)	Day 1	132.70±40.05	117.10±19.73	0.289	125.60±14.19	120.60±12.74	0.418
	Day 21	147.30±32.25	387.00±18.49	<0.001	132.00±15.25	362.8.0±28.28	<0.001
TG:HDL	Day 1	0.72±0.19	0.82±0.27	0.341	0.95±0.23	0.93±0.18	0.840
	Day 21	0.79±0.39	4.18±1.48	<0.001	1.08±0.54	3.26±1.44	<0.001

Table-2: Muscle fatigue in isolated Soleus muscles between male and female diabetic groups

Contractile properties of Soleus muscle	Group IIa (Male diabetic)	Group IIb (Female diabetic)	*p*
Maximum fused tetanic tension (N/g)	0.03±0.03	0.03±0.03	0.857
Maximum fused tetanic tension after the fatigue protocol (N/g)	0.01±0.01	0.01±0.01	0.496
Tetanic tension after 5 minutes of rest period following the fatigue protocol (N/g)	0.01±0.01	0.004±0.01	0.346

Table-3: Properties of muscle fatigue in isolated EDL muscles between male and female diabetic groups

Contractile properties of EDL muscle	Group IIa (Male diabetic)	Group IIb (Female diabetic)	*p*
Maximum fused tetanic tension (N/g)	0.09±0.07	0.08±0.05	0.640
Maximum fused tetanic tension after the fatigue protocol (N/g)	0.05±0.05	0.01±0.01	0.063
Tetanic tension after 5 minutes of rest period following the fatigue protocol (N/g)	0.01±0.01	0.004±0.01	0.533

DISCUSSION

Extensive studies have been conducted on the global epidemic of T2DM to discern its pathophysiology, progression of complications and to explore new management options. Several animal models have been devised over the years to study its pathophysiology. The animal model for induction of T2DM applied in our study was comprised of feeding High Fat Diet (HFD) with low dose STZ administration. This model is considered feasible due to its easy accessibility, cost effectiveness and that the induced disorder closely resembles the pattern of metabolic syndrome in humans. Some studies[10,11] have recommended a similar animal model to use low dose STZ (35 mg/Kg body weight) and HFD to develop the pathological state of T2DM in Sprague Dawley rats. Streptozotocin (STZ/2-Deoxy-2-([(methyl nitrosoamino) carbonyl]amino)-D-glucopyranose is a hydrophilic, β cell toxic glucose analogue. It is taken up by the β cells of the pancreas via GLUT 2 (glucose transporter). STZ impairs the oxidation of glucose and reduces insulin secretion and biosynthesis to alter blood glucose and insulin

concentrations. The cytotoxic effects of STZ on β cells are consequent to the alkylation of DNA and formation of reactive oxygen species (ROS).[12] These factors contribute in β cell death and development of type 2 diabetic state.

A rise in body weight and blood glucose of both male and female diabetic groups was found to be significant (p<0.001) at the termination of the study. Intra-myocellular accumulation of lipid droplets augmented the insulin resistance after intake of HFD. Comparable significant (p<0.05) weight gain and hyperglycemia had been revealed by Lawrence J Coppey[13] while determining peripheral neuropathy in high fat diet and low dose STZ induced female Sprague Dawley rats. The diabetic rats developed frank hyperglycaemia in our study. High fat diet contributed to it and STZ destroyed the β cells of the pancreas.

In the current study Soleus (slow) and Extensor Digitorium Longus (fast) muscles of both diabetic groups were evaluated on a data acquisition unit for parameters of fatigue. Maximum fused tetanic tension (MFTT) between male and female diabetic rats showed no significant change (p=0.857 for slow muscle and p=0.640 for fast muscle) among them in both slow and fast muscles. A study conducted to determine effects of gender on skeletal muscles of diabetic rats revealed that males had significantly (p<0.05) higher tetanic force development than females.[4] This is in contrast to our study. That study mentioned only segregated Soleus (slow) muscles for studying contractile parameters while we utilized both slow and fast fibres, slightly lighter weighing rats, and a lower dose of STZ (35 *vs* 60 mg/Kg body weight) which might have caused differences in results. Similar results compared to our study were observed by Imam *et al*[14] in Soleus and EDL muscles while assessing all contractile parameters including fatigue between male and female diabetic rats. They concluded that no gender-based differences occurred in contractile parameters of diabetic rats, though they used a higher STZ dose (65 *vs* 35 mg/Kg body weight).

MFTT after fatigue protocol was found to be insignificant (p=0.496 for slow *vs* p=0.063 for fast fibres) between both genders in case of slow and fast muscles. A study where levocarnitine levels and muscle functions were assessed in diabetic rats found that MFTT after fatigue protocol was significantly (p=0.020) reduced in diabetic rats[7], but there was no differences regarding gender. This reveals extinction of fuel reserves in skeletal muscles to bring about such reduction in tetanic tension. In our case, probably both genders lost similar amount of fuel reserves revealing similar changes in MFTT after fatigue protocol.

Athan G Dial *et al*[15] studied the effects of diabetes on muscle function of male and female human subjects and suggested significant impairment in muscle functions of both male and female diabetics. Our project has focused on the deteriorating effects of T2DM on the skeletal muscles during fatigue but, differences based on gender could not be substantiated.

Tetanic tension after 5 minutes of rest period following the fatigue protocol usually exhibits betterment in contractile function as the muscles get time to replenish their resources. Our study compared this property in both genders for slow and fast fibres where it was found to be insignificant (p=0.346 for slow *vs* p=0.533 for fast fibres), yet showed more reduction in female diabetic rats in both slow and fast muscles. Reduction in recovery from fatigue was observed in type 2 diabetic rats while studying their contractile functions, but differences in gender were not analyzed. Tetanic tension after 5 minutes of rest period following the fatigue protocol was reported[16] to be significantly decreased in diabetic rats while studying the effects of levocarnitine on endurance capacity in them. Increased fatigue in type 1 diabetics and lower muscle strength was established in a study[17] on human subjects with diabetic polyneuropathy indicating exhaustion of muscle reserves. This would have drastically deranged their tetanic tension after fatigue protocol and also tetanic tension 5 minutes after fatigue protocol.

Most of the data suggest enhanced fatigability of slow and fast muscles due to diabetes mellitus. Data on gender based differences in these properties is helpful, but scarce. Our study highlighted the importance of more gender based research that needs to be practiced in order to identify specific management options and prognosis of diabetes mellitus.

CONCLUSION

There are no differences in the parameters of fatigue between male and female diabetic rats for both Soleus and EDL muscles.

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Contribution of Authors

MA: Concept, Study design, Data analysis and interpretation, Final approval
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SM: Literature review, Draft manuscript, Critical revision
AR: Draft manuscript, Critical revision
SH: Critical revision
AJ: Data analysis and tabulation

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