

Original Article

Gender-effect on the contractile properties of skeletal muscle in streptozotocin-induced diabetic rats

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Abstract

In the present study, we studied the effect of streptozotocin-induced Type 1 diabetes on contractile properties of soleus muscle in female and young male rats. We hypothesized that the gender affects the contractile function in diabetic rats. Thirty-two Sprague-Dawley rats, male and female, three months old were divided into four groups: Female Non-Diabetic (FND), Female Diabetic (FD), Male Non-Diabetic (MND) and Male Diabetic (MD). Diabetes was induced by a single dose of 60 mg/kg body weight of streptozotocin in citrate buffer pH 4.5 by intraperitoneal route. At 4 weeks after of the dose animals were considered to be diabetic if they had glucose levels ≥ 20 mmol/L. Soleus muscle mass and twitch force were higher in MND than in FND; in male rats, the diabetes decreased the muscle mass in 34% and the twitch force decayed in 33%; while in diabetic females the muscle mass and twitch force decayed 15% and 10% respectively. Our results showed that the diabetes has gender-dependent effects on the muscle mass and maximal contractile force.

Keywords: Diabetes, Skeletal Muscle, Soleus, Sex-Difference

Introduction

Diabetes mellitus (DM) is a metabolic disorder that continues growing to be a major worldwide epidemic. The prevalence of diabetes is rapidly increasing from 171 million in 2000 to an estimated 366 million in 2030¹. There are two main types of diabetes: Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) and the prevalence of diabetes has been reported higher in men than women¹. However, the beta cell failure/death is the cause of development of T1DM² and T2DM³. Diabetes is characterized by clinical symptoms that include a deficit in the capacity for muscle force development, lower muscle mass, and reduced physical capacity, manifested as fatigue and therefore reduced quality of life. In T1DM, this alteration progressively reduces the physical performance of the patient in daily activities⁴⁻⁷. A loss of physical activity in diabetic patients often leads to increased

morbidity and mortality, as well as cardiovascular and respiratory diseases⁸. The muscle is the most important regulator of insulin-dependent circulating glucose and an impaired hormonal signaling has a deleterious effect on glucose uptake^{9,10}. Investigations on patients and animals with T1DM and T2DM models designed for studying the effect of diabetes on skeletal muscle showed that the muscle mitochondrial ATP production rate and expression of oxidative phosphorylation genes are decreased¹¹, there is a reduction in the production of force and loss of muscle mass^{6,12-14}. Moreover, proteomic profile alterations induced by diabetes have been reported in different studies, increasing the expression of stress response proteins¹⁵, altered expression in the glycolytic enzymes¹⁶, abnormal phosphorylation of ATP synthase¹⁷, increase of glycolytic/fast-twitch muscle fiber and glycolytic enzymes¹⁸, and cytoskeleton defects in the sarcolemmal dystrophin-dystroglycan complex¹⁹.

Although the streptozotocin (STZ)-induced T1DM effects on the muscle have been investigated, there is a lack of knowledge on the effects of gender or the diabetes-induced changes in the skeletal muscle in young patients. Recently, the National Institutes of Health (NIH) of the U.S. has suggested consider the sex as a biological variable in preclinical research^{20,21}.

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Table 1. Soleus muscle mass and contractile properties.

	Female		Male	
	FND (n = 8)	FD (n = 8)	MND (n = 8)	MD (n = 8)
Soleus muscle mass (g)	0.14 ± 0.01	0.12 ± 0.01	0.24 ± 0.02†	0.16 ± 0.01††
Twitch				
Peak Force (N)	0.55 ± 0.06	0.50 ± 0.04	0.83 ± 0.12†	0.56 ± 0.02††
Muscle quality (kN/kg)	3.72 ± 0.20	3.58 ± 0.25	3.59 ± 0.29	3.56 ± 0.10
Tetanus*				
Maximal Force (N)	1.29 ± 0.08	0.99 ± 0.10††	2.64 ± 0.11†	1.33 ± 0.14††
Muscle quality (kN/kg)	8.97 ± 0.40	6.97 ± 1.00††	11.28 ± 0.99†	8.02 ± 0.70††

All data correspond to mean ± SD. † p<0.05, non-diabetic female vs non-diabetic male. †† p<0.05, non-diabetic vs diabetic. *At 50 Hz of stimulation, kN = 1 x 10³Newton.

Previous studies show that for a better diabetes care and management is very important improve the knowing their physiopathology specifically gender-based differences, these would help a increasing in the quality of life of the diabetic patients^{22,23}. In a clinical study with diabetic elderly patients, the muscle strength was lower in diabetic than nondiabetic men, however there were not differences found in woman; meanwhile, the muscle quality was significantly lower in men and women with diabetes than those without diabetes^{14,24}. In STZ-induced experimental diabetes in rats, a study did not find differences in the contractile properties when female and male were compared, however this study lack a comparative analysis between diabetic rats versus healthy rats²⁵, this comparison is essential to evaluate the diabetes-induced effects in the skeletal muscle functional activity. In the present study, we performed a comparative analysis between females and males on the effect of the experimental diabetes in the mass and contractile properties of soleus muscle from young rats. Our results showed that the diabetes has effects gender-dependent on the mass muscle and maximal contractile force.

Materials and methods

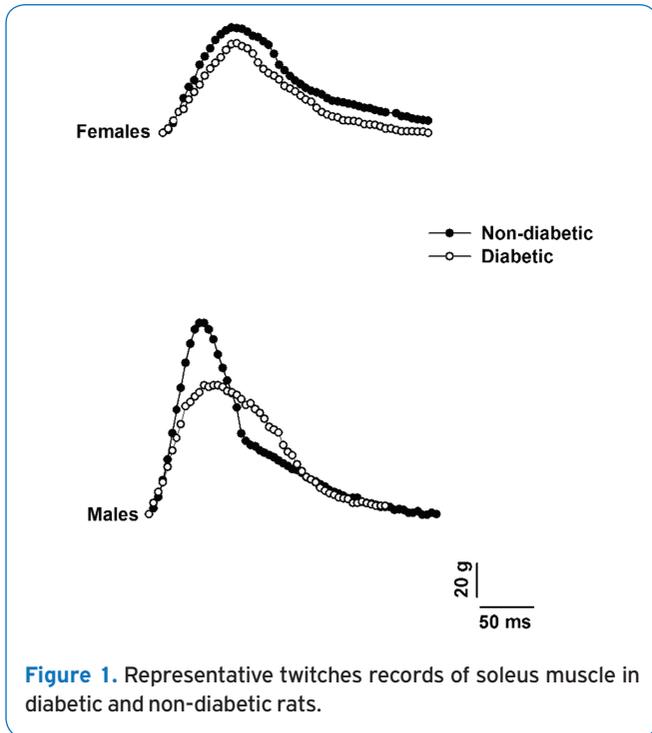
Treatment and care of animals

Animal care and experimental procedures were approved by the Ethics Committee of Colima University, using guidelines based on the Guide for the Care and Use of Laboratory Animals (US Department of Health, NIH). Thirty-two Sprague-Dawley rats, male and female, three months old were divided into four groups: Female Non-Diabetic (FND; body weight= 265±9 g; blood glucose level= 4.94±0.22 mmol/L; n=8), Female Diabetic (FD; body weight= 220±7 g; blood glucose level= 27.69±0.85 mmol/L; n=8), Male Non-Diabetic (MND; body weight= 275±10 g; blood glucose level= 4.95±0.25 mmol/L; n=8) and Male Diabetic (MD; body weight= 207±8 g; blood glucose level= 28.39±0.94 mmol/L; n=8). The diabetes was induced by a single dose of 60 mg/kg body weight of STZ in citrate buffer pH 4.5 by intraperitoneal

route²⁶. The animals in non-diabetic group received an equivalent volume of the vehicle. At 4 weeks after the STZ injection, the animals (female and male) were considered to be diabetic if they had glucose levels ≥20 mmol/L²⁷. Blood glucose was determined by the glucose oxidase technique in blood samples collected from the tail veins. All rats were provided with water and food (rodent laboratory chow, Purina; Composition: 23.9% Protein, 5% Fat, 5.1% Fiber, 7% Minerals), *ad libitum* and were maintained on individual acrylic cages (using a natural bedding, HER-BIO Purina) on a 12:12-h light-dark cycle with an average daily temperature of 24±1°C and average humidity of 60-70%.

Surgery and experimental conditions

Four weeks after the diabetes induction, all rats were subjected to surgical intervention to evaluate contractile activity of the soleus muscle. Rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The right soleus muscle was dissected free from the adjacent tissues, leaving the bone insertion and blood supply intact. The sciatic nerve was cut as far away as possible from its entry into the muscle. During surgery, saline solution (125 mM NaCl; 5.4 mM KCl; 1.05 mM MgCl₂; 1.8 mM CaCl₂; and 11 mM glucose, pH=7.4) was applied to protect the tissues. A transverse hole was made in the femur using a micro-drill (F.S.T.18000 - 17; Fine Science Tools, Foster City, CA, USA), and the distal tendon of soleus muscle was tied to a hook then each rat was transferred to a mechanical recording system consisting of a plate mounted on an inclined base that allowed the muscle to be placed perpendicularly to a load transducer (FT10; Grass Co., Quincy, MA, USA). The plate had two posts to hold the steel rod passing through the hole in the femur. The transducer was mounted on an actuator driven by a computer-controlled stepper-motor and wired to an A/D converter to allow display and storage of the force responses. The tendon hook was attached to the transducer. The sciatic nerve was placed on stimulating electrodes wired to a stimulator (S88; Grass Co.). During the experiment, the rat body temperature



was maintained at 37°C. The environmental temperature was 24°C. At the end of the experiments, the soleus muscle were excised from the animals and weighed on an analytical balance (Sartorius, Edgewood, NY, USA).

Mechanical properties measurement

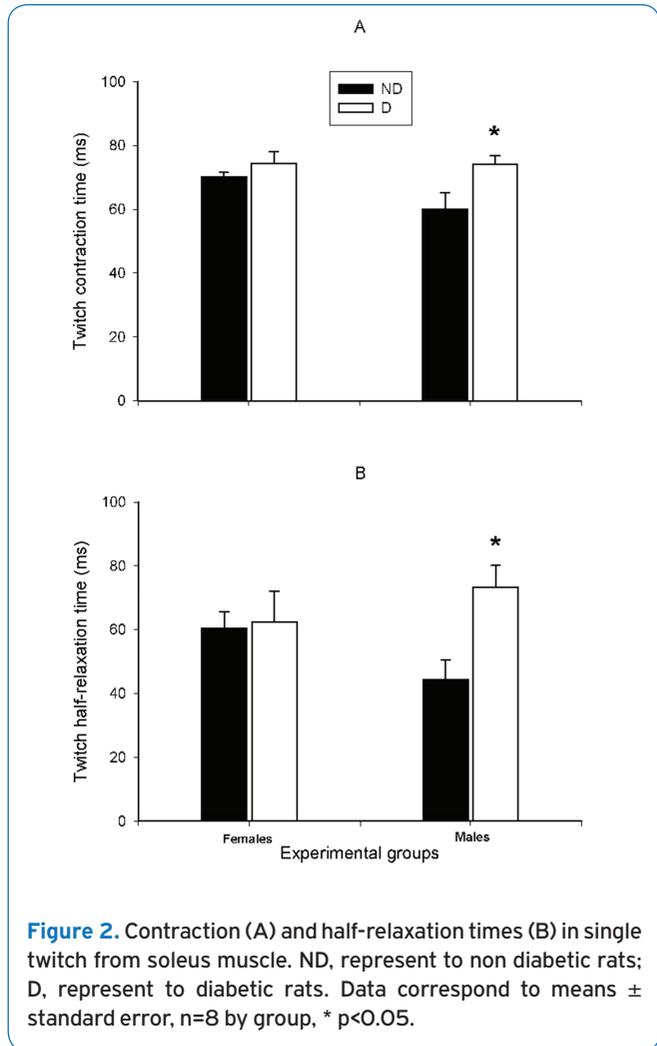
At several muscle lengths, single isometric twitches were elicited by supramaximal stimuli (3 volts applied) to the sciatic nerve until reached the maximal force; this length was defined as the optimal length (Lo). At Lo of soleus muscle, single twitches and tetani were recorded (5-100 Hz) elicited by motor nerve stimulation. The muscle was rested for 100 s between each record. Finally, the animals were euthanized by anesthetic overdose (150 mg/kg, intraperitoneally).

Mechanical analysis

The obtained tensions were expressed as force (N). The contractile function of skeletal muscle is dependent on the quantity of muscle mass and due a difference in skeletal muscle mass between healthy and diabetic rats, then for the comparison between groups was used the concept of skeletal muscle quality, defined as the maximal contractile force to muscle mass ratio¹⁴ (in our case soleus muscle mass) and expressed in kN/kg.

Statistical analysis

Comparative analysis between pairs was performed using a Student test with a 95% significance level. The different tensions of frequency were compared with an analysis



of variance of one factor and a post hoc Tukey's test. All differences were considered statistically significant with a $p \leq 0.05$. Statistical analysis was conducted with software Minitab Release version 12.

Results

The body weight loss after four weeks of the diabetes induction was higher in MD than FD group (MD, 29 ± 5 g; FD, 10 ± 4 g); in contrast in the same time period, the healthy rats gained body weight (MND= 24 ± 3 g; FND= 6 ± 2 g). These results showed that in healthy and diabetic rats body weight variation is gender-dependent. Soleus muscle mass and twitch force were higher in MND than FND ($p < 0.05$, data showed in Table 1). The diabetes significantly decreased 34% the muscle mass and 33% the twitch force in the male rats, while in diabetic females decayed 15% and 10% the muscle mass and the twitch force respectively (Table 1). When the twitch force was normalized to muscle mass, (skeletal muscle quality), there were not differences

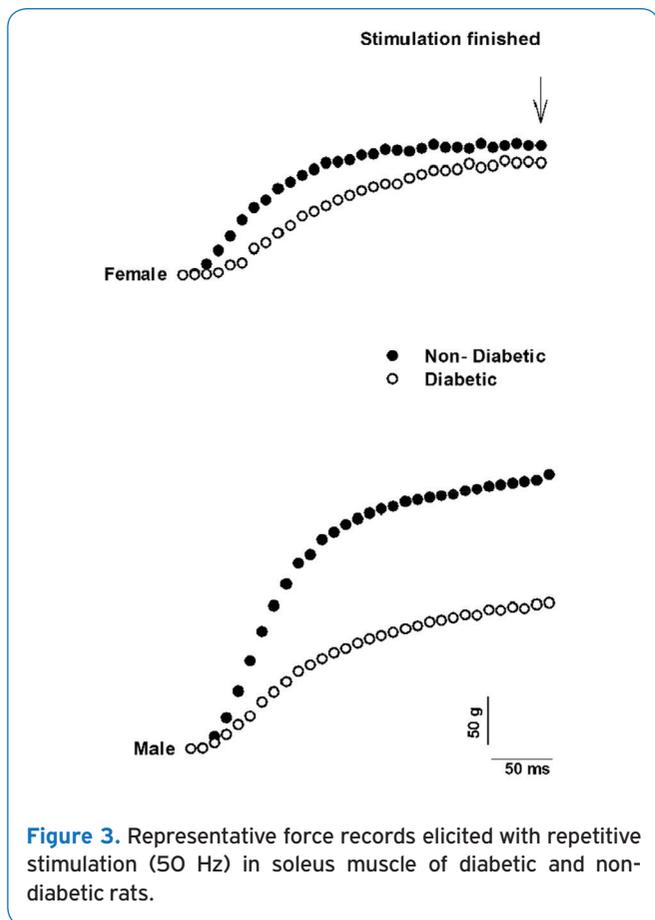


Figure 3. Representative force records elicited with repetitive stimulation (50 Hz) in soleus muscle of diabetic and non-diabetic rats.

between females and males in the studied groups. In addition, we studied the relaxation and contraction times of the soleus muscle and as shown in the Figure 1 and Figure 2, the diabetes did not affect the twitch contraction and the half-relaxation times in the females; however, in males, diabetes increased significantly the twitch contraction and the half-relaxation times ($p < 0.05$). In relation to the maximal tetanic force developed for the soleus muscle at different stimulation frequencies (Figure 3), the males significantly developed more tetanic force than the females ($p < 0.05$, Figure 4) and diabetes reduced force development in both, females and males. However, the effect of diabetes on the tetanic force development is higher in males than in females. The skeletal muscle quality was less in the diabetic rats (Figure 5), and the diabetes effect was significantly greater in males compared with the females ($p < 0.05$).

Discussion

We studied the effect of the experimental diabetes in the mass and contractile properties of soleus muscle of young rats from the point of view of sex-based difference. In the literature there few studies carried out to understand differences of gender in the deterioration of skeletal muscle

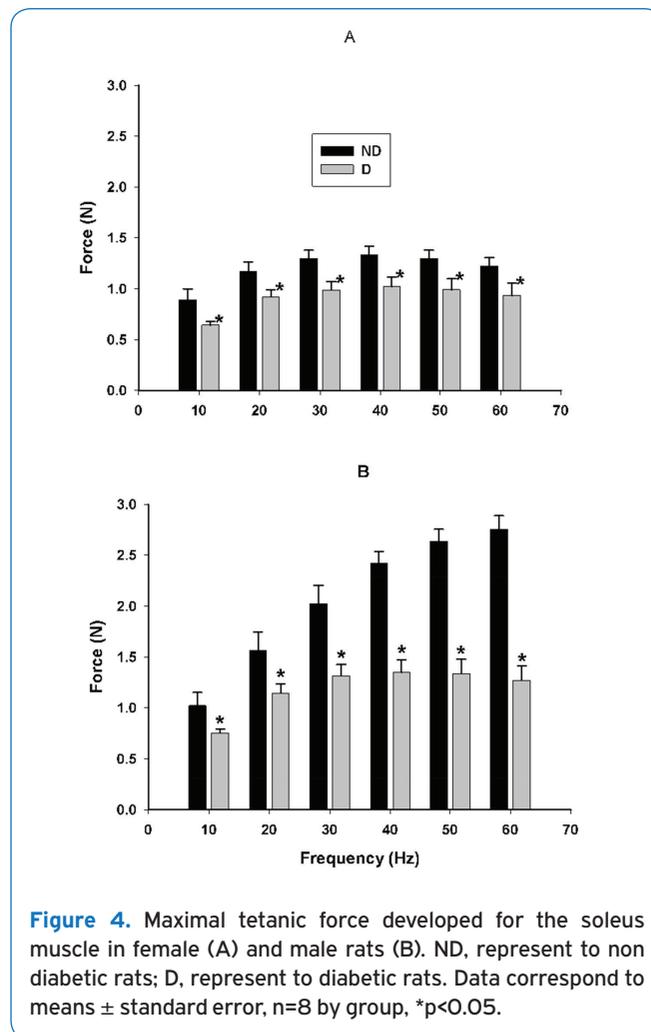


Figure 4. Maximal tetanic force developed for the soleus muscle in female (A) and male rats (B). ND, represent to non-diabetic rats; D, represent to diabetic rats. Data correspond to means \pm standard error, $n=8$ by group, $*p < 0.05$.

in STZ-induced diabetes. A study in diabetic persons with physical activity showed that there are not differences gender-based on strength and contractile properties²⁸, and T2DM older adults showed a significantly decrease in the absolute muscle strength in men but not in the woman. Moreover, the muscle quality was significantly lower in both, women and men with diabetes than without diabetes¹⁴. The experimental diabetes in young rats showed that absolute twitch force decayed significantly in male, however, in female it not changed significantly. Also, we demonstrated that absolute tetanic force and muscle quality decayed significantly in diabetic females and males, but the males are more susceptible to diabetes-induced effect on the muscle than females. Although mechanisms underlying to gender differences in muscle performance during diabetes are still poorly understand, peripheral fatigue can play a key role, recent study showed that diabetic male have higher peripheral fatigue than female²⁹.

In the diabetic males, the relaxation-half and contraction times of soleus muscle increased respect to non-diabetic males, those is consistent with others studies^{30,31}. On the

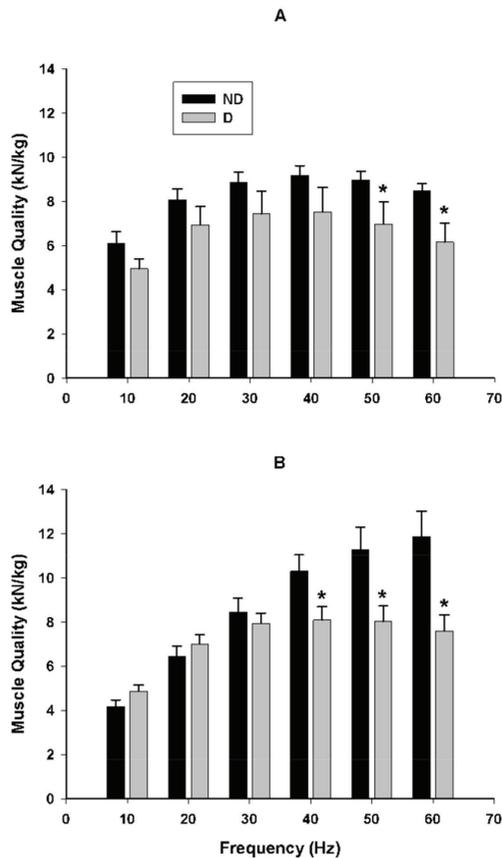


Figure 5. Soleus muscle quality in female (A) and male rats (B) to different frequency. ND, represent to non diabetic rats; D, represent to diabetic rats. Data correspond to means \pm standard error, $n=8$ by group, $*p<0.05$.

other hand, the relaxation-half and contraction times in the soleus muscle were not altered significantly for diabetes in the females. Although our results were found in the slow soleus muscle, which is mostly the oxidative in rodents and humans, similar results were found in a study using the fast glycolytic extensor digitorum longus muscle (non-oxidative) of non-diabetic and diabetic female rats³². The changes showed in the contraction time in diabetic males, that could have been caused by the increased sensitivity to Ca^{2+} of the contractile properties of skeletal muscles³³ and a Ca^{2+} diminished release induced by an increase in the calsequestrin expression^{34,35}, the main calcium binding protein of the sarcoplasmic reticulum, while the augmented in the relaxation-half time could be caused by impaired Ca^{2+} uptake by sarcoplasmic reticulum³⁶. Ca^{2+} -ATPase activity and Ca^{2+} uptake in skeletal muscle are gender-affected in diabetes³⁷, this deregulation can explain relaxation and contraction times differences in the current study.

In diabetic males, the increased in the relaxation-half and contraction times, as well as the reduction in the production of soleus muscle force, also may be related to

an increase in the proportion of slow oxidative fibers at the expense of fast oxidative glycolytic fibers³⁸. In the cardiac muscle, the contractility is sex-dependent and is related to different protein levels and/or Ca^{2+} -handling functioning systems³⁸, however, in skeletal muscle are required further investigations. Although the studies reported in literature are limited, there are evidence than female skeletal muscle has less fast fibers amount in comparison to male^{39,40}; this difference could result beneficial for female in conditions as the diabetes, it supporting that diabetes do not have significant effects on the relaxing and contraction times in soleus muscle.

The muscle mass and the contractile properties of the soleus muscle in male and diabetic female could be explained by the reduction in the testosterone levels in male rats with STZ-induced diabetes⁴¹. Studies suggest that the gender effect and estrogen on skeletal muscle depends of the type of muscle. Recently, it was shown that in soleus muscle (slow muscle) of pregnant rats (high estrogen levels) increase the fatigue resistance with regard to non-pregnant rat⁴²; while estrogen and gender did not affected the fatigue resistance in EDL muscle (fast muscle)⁴³. Therefore, our results in the soleus muscle could be explained by the estrogen ability to reduce oxidative stress and promote the formation of cross-bridges⁷, but is necessary more studies.

Metabolic differences between male and female muscles may contribute to observed differences in the strength development during this study. A recent study reported a decrease in the alpha-enolase expression pattern in skeletal muscle of diabetic male rats and an increase in female rats, as is known this enzyme plays an important role in the glycolytic metabolism to produce energy (ATP), essential for muscle contraction⁴¹. Furthermore, it is known that in the diabetes the soleus muscle (mainly composed of slow fibers) accumulates twice of the intramyocellular lipids in comparison with the tibialis muscle (fast fibers)⁴⁴. This suggests that lipids could be used by diabetic skeletal muscle as an alternative source of energy and could be another possible explanation for our results. From the above, during the diabetes the slow muscle would have a bigger availability of lipid reserves to produce ATP. Also, studies performed in rats have shown that lipid oxidation is higher in female skeletal muscles than in male muscles, which may explain why diabetic skeletal muscle has greater functional performance in females than in males^{45,46}.

Another factor that should be considered is oxidative stress due to a previous study it has been associated with inactivation of the calcium channel voltage-dependent and Ca^{2+} ATPase⁴⁷, as well as a decrease in the generation of force⁴⁸. Previous reports in skeletal muscle show that oxidative stress is higher in male than female^{49,50}, this suggest that oxidative status differences gender-based would be crucial to the decline in the functional activity of the skeletal muscle in chronic diseases as the diabetes. However, more research is necessary to evaluate the effect of gender on oxidative stress in skeletal muscle generated during diabetes.

Skeletal muscle is fundamental to regulate insulin-dependent blood glucose level. Previous studies have reported that the insulin level is greater in male than in

healthy female rats⁵¹, and moreover, females have a better adaptive response in skeletal muscle to a drastic change of insulin by GLUT-4 regulating expression⁵², some studies have reported that GLUT4 expression is higher in female than male^{53,54}. However, this sex-based mechanism has not been fully established in diabetes.

In conclusion, our study shows experimental evidence about the differential gender effects on the contractile mechanical properties of the soleus muscle of young diabetic rat. Therefore, this study on gender-based is relevant in improving the understanding about the functional alterations presented in the diabetic patients and, therefore, in future occasions they can receive more appropriate medical care and have a better quality of life.

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