The Effects of Six Weeks Endurance Training on Soleus and Extensor Digitorum Longus Myonuclear Number in Diabetic Male Wistar Rats

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Received: 12 April 2017

Accepted: 21 November 2017

Published in August 2018

Abstract

Objective: The importance of skeletal muscle as the largest metabolic tissue in diabetes remains more unknown than other metabolic tissues of the body. The purpose of this study was to evaluate the effects of six weeks endurance training on the soleus and extensor digitorum longus (EDL) myonuclear number in diabetic male wistar rats.

Materials and Methods: In this study, 40 male wistar rats about 10 weeks old and weighing 200-250 grams allocated randomly in four groups of diabetic training (DT), diabetic control (DC), healthy training (HT) and healthy control (HC). For induction of diabetes, DT and DC groups were intraperitoneally injected by streptozotocin (STZ), and the training groups performed incremental endurance training on the treadmill for six weeks. Fourty eight hours after the last training session, all rats were killed and tissue samples of soleus and EDL muscles were removed and fixed in 10% buffered formalin. The sections were prepared with six µm thickness and stained with hematoxylin–eosin. The myonuclear numbers were counted in prepared plates by randomly style at the ten field microscopy. Data analysis was done with One-way and two-way ANOVA and Tukey's post hoc test.

Results: Our findings showed that myonuclear number in diabetic groups was lower in both soleous and EDL muscles (*P*-value: 0.0001). furtheremore in DT and HT groups, the number of nuclei increased significantly (*P*-value: 0.0001).

Conclusion: Endurance physical activities as a non-medicinal strategy can play an important role in maintenance of the structure and the function of skeletal muscles and thereby improving the quality of life in diabetes.

Keywords: Diabetes mellitus, Endurance training, Soleus, Extensor digitorum longus

Introduction

During the last three decades, the number of adults with diabetes has doubled worldwide(1). Diabetes is a chronic disease which has remained a problem for the world health organizations (2).

Skeletal muscle is a striped tissue which has an important role in metabolism, movement and general health body, as the largest metabolic organ of the body (3). Its importance in diabetes comparing to other

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metabolic tissues has remained unknown. The previous studies have shown that diabetes causes physiological, functional and morphological undesirable changes in skeletal muscles which is known as myopathy (2). Contraction weakness, fiber type changing, decrease in oxidative activity and peripheral insulin resistance occur in the involved muscles (4).

Nucleus is the largest organelle in eukaryotic cells (5). The number of nucleus in muscle cells are very important since determine the amount of DNA for genes transcription (6). Distribution of nuclei is not random in muscle cells. Also, it is important for transduction activities in large muscle cells. The best distribution is when there is the greatest average distance between nuclei and the worst distribution would be to cluster all of the nuclei in one place that lead to shorting distances between the nuclei which requires longer distances to move to the end sections of the cell (7).

In several of muscles diseases, number and distribution of myonucleus change that leading to the muscle dysfunction (8,9). The numbers of myonucleus depends on the type of muscle fiber. Fast twitch fibers have fewer nuclei and consequently, have a larger nuclear domain in per micrometer of muscle fiber. This difference in myonucleus number as well as nuclear domain is attributed to the difference in metabolic needs and protein expression between slow and fast twitch fibers (10). It has been also reported that the nucleus of fast-twitch muscle fibers, such as extensor digitorum longus (EDL) is oval and aligned with the longitudinal axis of the fiber, while in the slow-twitch muscles such as soleous nucleus have a rounded shape (7).

Positioning of myonucleus in fast-twitch muscles is more regular, but in slow-twitch it is a more random pattern. The slow-twitch patterns of positioning of nuclei may facilitate inter-nuclear relationship to regulate and coordinate of protein expression (11).

There are evidences to support the role of regulating the number of myonucleus during reconstruction muscles in response to injury, adaptation and disease. These studies have shown that muscle hypertrophy occurs by formation of new nuclei through the combination of myogenic in adult myofibers, whereas muscle atrophy and disease probably appear to be associated with a reduction in the number of nuclei through mechanisms similar to apoptosis (12). It has also been shown that atrophy has a more effect on slow-twitch rather than fast-twitch fibers (10).

Exercise and physical activity are the non-medicinal strategies in treatment of diabetes. There is a significant relationship between the number of myonucleus and cross section muscle fiber. The comparison of ten elite powerlifting athletes with nonathletic subjects showed that the number of myonucleus in each muscle fiber at the trapezius muscle was significantly higher in elite athletes rather than non-athletes (13).

One of the diabetes effects on muscle fibers is atrophy and probably a decrease in the number of myonuclei (14,15). In a study after a diabetic rats with Streptozotocin (STZ) was shown that running on treadmill for four weeks cause to activation of satellite cells (16). The satellite cells can produce the new muscle nuclei (17). Therefore, physical activities may reduce muscle atrophy and weakness in diabetes myopathy, through activating the satellite cells and results in increasing muscle nuclei.

Some studies showed that 12 weeks of resistance training caused a significant increase in muscle nuclei in type 1 and 2 fibers in vastus lateralis muscle and no differ between two types of muscle fibers(18). Also, 12 weeks of aerobic exercise on cycle ergometer resulted in a significant increase of the myonuleous number in type 1 fibers, but did not found significantly change in type 2 muscle fiber (19,20).

whereas many studies investigated the relationship between the muscles mass and function, growth, development and muscles improvement as well as metabolic activity in different types of diabetes mellitus. However

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no study investigated the changes of muscle nucleus and possible role of physical activity in diabetes. The aim of this study was to investigate the changes of myonuleous and potential effects of physical activity in diabetes myopathy.

Materials and Methods

The present study was an experimental study which performed with before-after design and control group. In this research, 40 wistar rats with ten weeks old and 200- 250 g weight were divided randomly to 4 groups, diabetes training group (DT) which include 10 diabetic wistar rats and from 12th weeks of living performed incremental endurance training on the treadmill for six weeks and 5 sessions each weeks. Diabetic control (DC) includes 10 diabetic wistar rats which didn't have any exercise program. The healthy training (HT) includes 10 wistar rats and similar to DT group were performed treadmill exercise and the healthy control (HC) group which consists of 10 wistar rats with no exercise program.

All of the rats were kept in an animals care place at research center of Lorestan medical science university. Rats were kept in the average temperature 22±2 C and on-off light cycle 12:12 in special cages made of polycarbonate. The food for rats was procured from pars domestic feed company and all of them had access free to rat special water and food. In all of the study process one person manipulated and displaced them. In this study working with animals were behaved morally confirmed by moral committee of Lorestan medical science university and the principles of the International Association for the Study of Pain (IASP).

After 12 hours strip of food, the injection of streptozotocin (STZ) 50mg/kg solved in 0.5 mol/L fresh citrate buffer with 4.5 PH intraperitoneally was done. The buffer citrate was also injected to healthy non-diabetic rats. After 48 hours the rats with higher blood glucose levels of 300 mg/dL were considered as diabetic. To ensure that blood glucose did not return, at during of the six weeks and end of the exercise program, the blood glucose levels were measured.

Exercise protocol

The endurance training was performed with moderate intensity (50-55% of maximal oxygen consumption). The exercise groups did training protocol on the treadmill according to the table 1 for six weeks and 5 sessions each week.

After 48 hours of the last training session, all of rats were anesthetized by carbon dioxide gas and killed. It should be noted that in order to count the number of muscle nuclei, 6-8 samples from each group were evaluated. Then tissue samples of soleus and EDL muscles were removed and fixed in 10% buffered formalin. Transverse and longitudinal sections were prepared with six μm thickness and stained with hematoxylin-eosin method. The myonuclear numbers were counted in prepared plates by randomly style at the ten field microscopy with × 40 magnifications by Olympus optical microscope equipped with camera and digital imaging device (BMZ-04-DZ- Behin Research company) and photomicrographs were obtained with a clarity of 12.1 megapixels.

It should be noted that the ethical code of this research is LUNS.REC.1395.170 from the Lorestan University of Medical Sciences.

Data analysis was performed using SPSS software version 21 to describe the data and draw charts and tables used from descriptive statistics. One-way ANOVA and Tukey post hoc test to compare the groups were done. Two-way variance analysis was used to evaluate the interactive effect of diabetes and training. The significance level was considered as $P< 0.05$.

Table 1. Endurance training protocol

Variable	1th week	2th week	3th week	4th week	5th week	6th week
Training duration(minute)	10	20	20	30	30	30
Treadmill speed(m/min)	10	10	15	15	18	18

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Results

Figure 1 showed the mean blood glucose during six weeks of endurance exercise. As shown, blood glucose levels throughout the training period in diabetic groups were greater than 300 mg / dL, which was considered as a diabetic threshold.

In all of the studied groups, the number of muscle nuclei in the slow-twitch muscle (Soleus) was significantly higher than the fast -twitch muscle (EDL) (Table 2).

As its shown in Table 3, the number of myonuclear in the diabetic groups was significantly lower than their control groups in the both soleus and EDL muscles (*P*-value: 0.001). Therefore, diabetes has reduced the number of muscle nuclei in the mentioned muscle. Moreover, in the HT and DT groups that performed endurance training, the number of myonuclear increased significantly (*P*-value: 0.001). This increase is shown in both slow-twitch (soleus) and fast-twitch (EDL) muscle (Table 3).

Also, the effects of interactive diabetes and endurance training on two soleus and EDL muscles were investigated by two-way ANOVA and there are a significant difference in the soleus (*P*-value: 0.004) and EDL (P-value: 0.017).

Discussion

Diabetes Myopathy is one of the complication of diabetes mellitus that can alter the structure and function of skeletal muscle. Diabetes induced muscle damage can cause muscle fibers metamorphosis and death (21). Histological aspects of our study showed that diabetes reduces the number of myonuclear in the soleus and EDL muscles. Since muscle nuclei are important in the normal functioning of the muscles, such as protein synthesis, gene expression, intracellular transfer, cell division, migration, differentiation, fertility and polarization (22,23). Therefore the reductions of muscle nuclei in diabetes destroy the normal functioning of the muscle. This dysfunction occurs in both slow and fast twitch muscles.

The complications of diabetes myopathy are atrophy, muscle weakness and decrease of the cross-section area in muscle fibers (14,15), which the significant decrease in studied muscles myonuclear is due to diabetic induced atrophy.

One of the reasons for diabetic-induced atrophy is the accumulation of fat in muscle

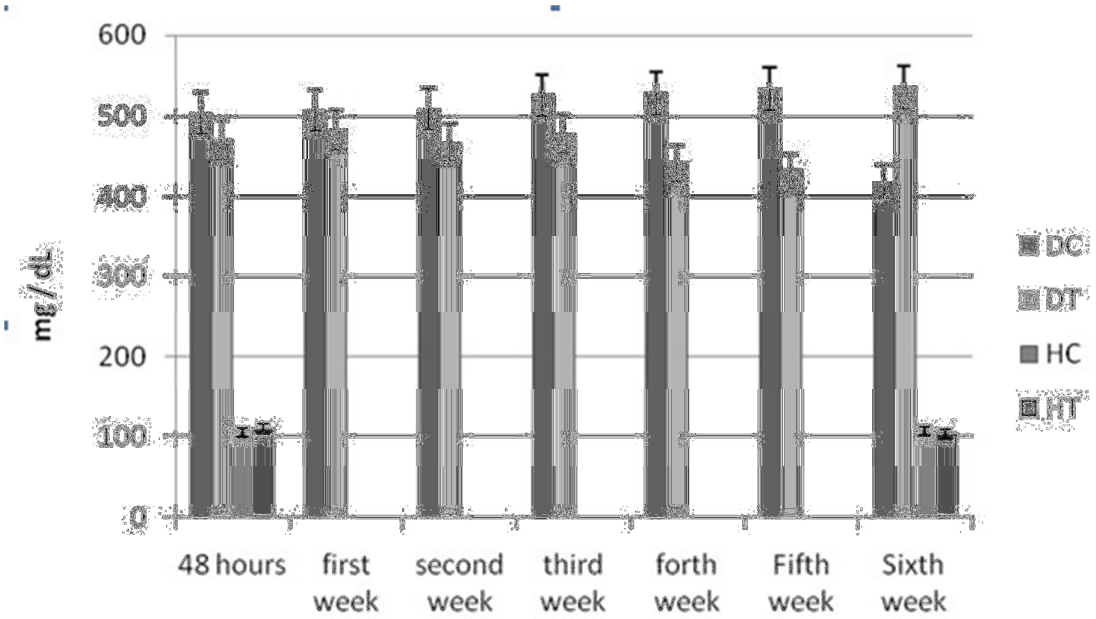

Figure 1. The mean of blood glucose during 6 weeks endurance training

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Table 2. Comparison of the myonuclear number in soleus and EDL muscles

Group	Mean difference	Standard deviation	*P*-value
Healthy control (HC)	20.025	15.991	0.001
Healthy training (HT)	9.400	16.881	0.001
Diabetic control (DC)	24.100	11.336	0.001
Diabetes training (DT)	28.300	6.436	0.001

Table3. Comparison of groups for the number of myonuclear in EDL and Soleus muscles (Tukey test)

Muscle	Groups		Mean difference	Std. error	Upper bound	Lower bound	*P*-value
EDL	HC	HT	15.100	2.395	-8.88	-21.32	0.001
		DC	10.800	2.395	17.02	4.58	0.001
		DT	5.600	2.395	11.82	-0.62	0.094
	HT	DC	25.900	2.395	32.12	19.68	0.001
		DT	20.700	2.395	26.92	14.48	0.001
	DC	DT	8.200	2.395	1.02	-14.42	0.015
Soleus	HC	HT	4.475	1.819	0.25	-9.20	0.019
		DC	6.725	1.819	11.45	2.00	0.002
		DT	2.675	1.819	2.05	-7.40	0.458
	HT	DC	11.200	1.819	15.92	6.48	0.001
		DT	1.800	1.819	6.52	-2.92	0.756
	DC	DT	9.400	1.819	-4.68	-15.92	0.001

fibers (24). In the present study, structural changes in the soleus and EDL muscles of diabetic groups were recognized which may be due to the entry and accumulation of lipids in the myofibers.

Another finding of the present study is the accumulation of muscle nuclei at a special point in the diabetic muscles. This pattern of nuclear distribution disturbs the normal and desirable muscle function, because as above-mentioned, the proper distribution of the nuclei and their spacing are essential for natural muscle fiber function and the transfer of substances and signals that needed in the muscle fibers.

In this research, it was shown that in the DT group, the number of myonuclear was significantly higher than control group. These findings, which are consistent with the findings of Charifi and et al (25), Fujimaki and et al (16) and Snijdersand et al (18) can be attributed to increasing the cross-section of muscle fiber and increased the number and activity of satellite cells. Therefore, endurance exercise with increasing the number of myonuclear in diabetic muscles can provide the improvement of muscles structure and function.

Fry and et al (2014) in their study showed that 12 weeks of aerobic training on a cycle ergometer resulted in a significant increase in the number of myonuclear in type 1 muscle fibers, while did not reported this result in the type 2 muscle fibers (19).

The pattern of distribution and regular distances of nuclei is essential for facilitating the relationship between the nucleus to regulate and coordinate the protein expression and other cells natural functions (11). In diabetes myopathy, this pattern is disrupted. The physical activities can be effective in maintaining muscle structure and function in myopathic diabetes by changing the number and distribution of myonuclear.

Conclusions

Overall, according to the results of this study, as well as the important role of muscles in quality of life, people who suffer from diabetes myopathy, can by doing physical activities, especially endurance training as a non-medicinal strategy, help to the maintenance of muscle structure and function.

Acknowledgments

This research is a part of a doctoral thesis in the field of exercise physiology, which has been approved by the Lorestan University. We would like to specifically thank the personnel of Razi

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Laboratory in Khorramabad and all those who helped us for accomplishment of the present study.

Funding

The authors received financial support for the research from Lorestan University.

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