The Effects of Six Weeks Endurance Training on XBP-1 Protein in the Diabetic Male Wistar Rats Sciatica Tissues

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Abstract

Objective: The aim of this study was to investigate the effect of endurance activity on XBP-1 protein content in sciatica tissue of diabetic male Wistar rats.

Materials and Methods: The experimental design was a post-test design with control group. In this study, 32 male Wistar 10 weeks old rats with the mean weight of 253.16 (±10.16) grams were randomly divided into four groups: healthy control, healthy exercise, diabetic control and diabetic training group. An intraperitoneal injection of STZ (45 mg / kg) solution was used to induce diabetes after 12 hours fasting, 48 hours after STZ injection, the moderate-intensity endurance protocol was performed for 6 weeks. Twenty-four hours after the last training session, the mice were exposed and their sciatic nerve was extracted. The amount of XBP-1 protein was measured by ELISA method. For statistical analysis, SPSS software and two-way ANOVA with repeated measure and independent T-test were used.

Results: Data analysis showed that endurance activity had an effect on the amount of XBP-1 protein and reduced the protein content, so that the difference was significant in the diabetic training group compared to the diabetic control group (*P*-value: 0.007).

Conclusion: These findings indicated that moderate aerobic exercise has a significant effect on the amount of XBP-1 protein in healthy and diabetic rats.

Keywords: Endoplasmic reticulum, Diabetes, Sciatic nerve, XBP-1 protein

Introduction

The complex network of environmental and genetic risk factors causes diabetes (1). The endoplasmic reticulum (ER) is an intracellular organelle that is the center of protein maturity and folding also plays a very important role in calcium homeostasis (2). Stresses that alter the ER of the homeostasis may interfere with the flaking of proteins, leading to accumulation of unfolded protein response (UPR) or proteins that have not been properly absorbed. This can lead to cell damage (3). An increasing number of evidence suggests the role of ER stress in the pathogenesis of diabetes, and probably the primary signaling pathways are due to ER stress and reactive oxygen species (ROS) production (4). ER stress is associated with changes in the immune system and metabolic

abnormalities that play an important role in type 2 diabetes and its complications (3). The X-box Binding Protein-1 (XBP-1) is an important marker for ER stress (4). The reactionary response of the ER to endoplasmic stress is matching functional capacity with cellular need and to improvement these ER induced disorders (5). The XBP-1 protein, which continues to signal the transmembrane protein Inositol-requiring protein 1α (IRE1α), is a major component of the basic regulation of the family of basic leucine zipped protein (bZIP) (6). This family includes transcription factors involved in the ER, which has a wide range of effects on the physiologic and pathologic function (7). When stress occurs in the ER, IRE1α is activated by phosphor, which acts as a stress sensor and activates the signaling pathway (8).

A recent study showed that endoplasmic stress is associated with changes in the immune system and metabolic disorders, and it showed that the ER stress is more likely to be involved in type 2 diabetes and its complications (6). When this part of the cell suffers from a disorder (stress), the patient's blood sugar control is difficult and the patient experiences dyslipidemia, insulin resistance, oxidative stress and inflammation (9). The findings suggested an interesting link between two well-known molecular processes, the inflammation and inappropriate performance of the ER involved in metabolic diseases, and suggests that targeting this relationship can lead to new therapies (10). In obesity, the ER could not start an intracellular signaling cascade that responds to UPR and reduces the stress of the ER. This study suggests that obesity-related inflammation can disrupt UPR responses and thus disrupts the functioning of the ER (9,11).

In previous studies showed that endurance training has anti-inflammatory and anti-oxidative effects (12). Recent studies have been done to investigate the stress of the ER in the muscle, indicating that UPR activity in the skeletal muscle increased and induced endoplasmic stress after 4 weeks of endurance training. Reported UPR control and homeostasis plays a very key role in reducing stress occurring in the endoplasmic network (13). Researchers are looking for alternative ways to prevent or treat fewer complications in diabetic patients. The neurodegenerative adaptations resulting from exercise training are very useful for preventing and treating diabetes and diabetic neuropathy (14). According to the results of these studies, excessive expression of XBP-1 may lead to a deterioration in the homeostasis of the ER and thus to progress in diabetes and diabetes-induced neurodegeneration. The present study aims to use endurance training as a non-drug strategy in diabetic rats on XBP-1 protein levels in male wistar diabetic rats.

Materials and Methods

The present study was an experimental before-after design. In this study, 32 male wistar rats 10 weeks old with the mean weight of 253.16 (±10.16) grams were selected as the studied sample from the Animal Care Center of Lorestan University of Medical Sciences with the ethical code of LU.ECRA.2017.8. All animals were kept under controlled environmental conditions at an average temperature of 22 (± 2°) C, a light-dark cycle of 12:12 hours, with free access to water and special diet of the mouse. After two weeks familiarity with the laboratory environment and reaching a mean weight of 330.7 (± 3) gr, the subjects were randomly assigned to four groups of 8 diabetes mellitus, control diabetes, healthy subjects, and normal control group. During the identification stage and adapted to laboratory conditions, treadmills, and manipulation, the animals walked on a treadmill five days a week for 10-15 minutes and at a speed of 5 – 10 m / min.

Streptozotocin (STZ) diabetes induction: After 12 hours of food deprivation, by intraperitoneally injected STZ (Sigma, St. Louis, MO); 50 mg / Kg dissolved in fresh citrate buffer (0.5 mol / L); pH (5.0) diabetes induced (15). Non-diabetic animals were also injected with a volume equivalent of citrate

buffer. About 48 hours after the injection, a small drop of blood was placed on the glucometer and a tape was read by the glucometer (German company Roche), and a rat with a blood glucose level higher than 300 mg / dl, were included in the study as diabetic animals (16).

Two weeks after induction of diabetes, the endurance training protocol was performed for six weeks. In all sessions, the exercise was held at the end of the animal's sleep cycle between the 16th and 18th hours (17). In the present study, the moderate exercise intensity (55-50 Maximum Oxygen Consumption) was physiologically used (17). The activities groups were subjected to moderate intensity exercise for five days a week for a period of six weeks, with three days of exercise and one rest day. The velocity and duration of the revolving drill gradually increased (Table 1) (17). Also, no exercise shocks were used during the endurance training program and, if necessary, using hands or creating an acoustic stimulus on the treadmill rails, the animals were forced to continue training.

Laboratorial methods

Expression and extraction of sciatica tissue was performed 24 hours after the completion of the endurance training protocol. Animals were anesthetized by intraperitoneal injection of a combination of ketamine (40 mg / kg) and xanthine (8 mg / kg), and the sciatica tissue of the animals was treated with external posterior fossa of the sciatic nerve from the ischial nerve to the nerve bundle sciatica was cut and separated apart. The tissues were then stored at 70 ° C for negative analyzes. To evaluate the expression of XBP-1 in each group, molecular testing was performed by ELISA method. When the intracellular target molecule is poured into the wells, the monoclonal antibodies that are attached to the wells are already connected to their own sites. Secondly, by secondary antibodies, which are biotin and streptovudine conjugate The Horse Radish Peroxidase (HRP) enzyme forms a complex. Unconjugated supplements are removed from the environment and added to the H2O2 and TMB medium as a substrate. The HRP enzyme, which is an oxygenase, consumes added H2O2 and in this case the TMB is converted to blue. Finally, stop the whole reaction by stopping solution and read the solution OD at 450 nm. In this study, a biomagnetic kit was constructed in the USA with a sensitivity of 0.054 ng / ml.

Statistical analysis

For statistical analysis, SPSS software 19 and two-way ANOVA with repeated measure and independent T-test were used for post hoc test.

Results

At the onset of the exercise program, blood glucose levels significantly increased 48 hours after streptozotocin induction of diabetes in diabetic rats (*P*= 0.0001), and after 6 weeks of endurance training, there was a significant difference blood glucose levels between healthy subjects and diabetic rats (*P*= 0.0001). Also, at the end of the exercise program, the blood glucose level in the diabetes group was significantly lower than the control group for diabetes (*P*= 0.001), (Figure 1).

The initial weight of the groups did not differ significantly (*P*-value:0.05), but at the end of the study, the mean weight changes of the diabetic control group in the healthy control group and the diabetic group were significantly lower than the control group (*P*-value: 0. 003 and 0.004). (Figure 2)

Figure 3 showed the amount of XBP-1 protein in different groups, the level of XBP-1 protein in the diabetic control group is higher than the

Table 1. Continuous aerobic exercise protocol

Variable	First week	Second week	third week	fourth week	fifth week	sixth week
Duration (minutes)	10	20	20	30	30	30
Treadmill speed (m / min)	10	10	15	15	18	18

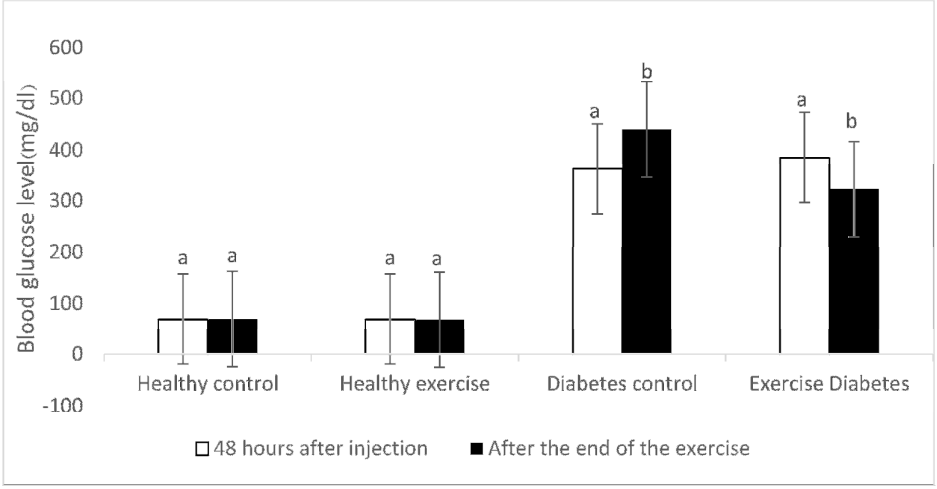

Figure 1. The difference of the blood glucose level in studied groups

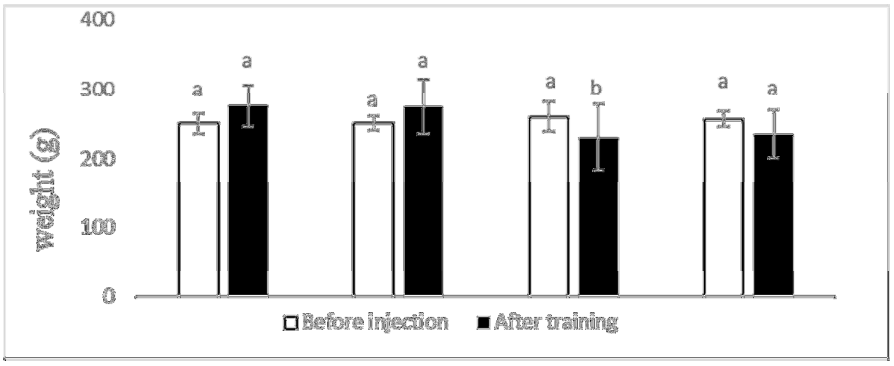

Figure 2. The difference of the weight in studied groups

rest of the groups, which is significantly different (*P*-value: 0.007) (Figure 3).

It has been shown in the post hoc test that endurance training reduced the amount of XBP-1 protein in training groups compared to non-exercised. The effect of diabetes has also been shown to increase this factor in the sciatic nerve. In the diabetes training group the amount of this protein decreased in comparison with the diabetes control, which was significant (*P*-value: 0.007). There were significant differences in healthy control and diabetes control groups (*P*-value: 0.001).

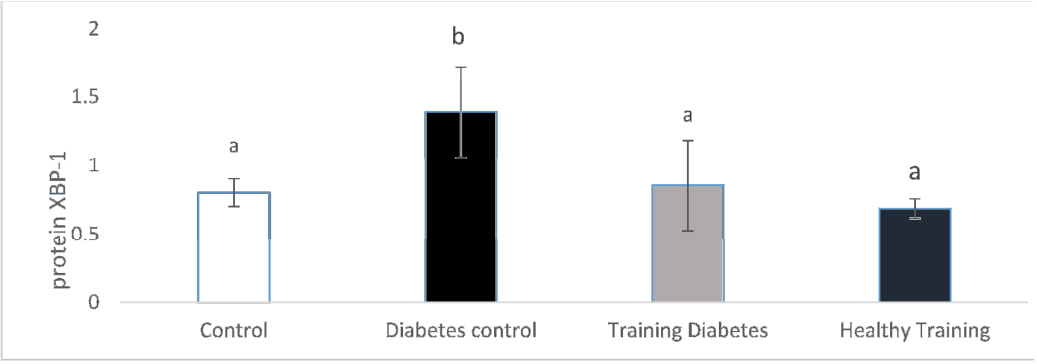

Figure 3. The mean of XBP-1 protein in the sciatic nerve

Discussion

The purpose of this study was to investigate the effect of six weeks of endurance training on XBP-1 protein in the sciatic nerve region of diabetic rats. This study showed that diabetes increased the amount of XBP-1 protein in the sciatic nerve. Previous studies have shown that the goal of initiating UPR is to re-establish homeostasis and normal ER function and compromising mechanisms that result in the expression of genes that are able to increase the amount of erosion of the ER protein (18,19). When the initial stimuli that cause the protein to be eaten in ER are long or over, the UPR's compromise mechanisms fail and cell death occurs through apoptosis. It is also known that ER stress can induce autophagia (8,20). The XBP-1 protein is furthered by the IRE1 signaling produced and contributing to the endometrium hemostasis (21). Zhang et al, showed that obese, high-fat dietary rats had endoplasmic stress in the hypothalamus and peripheral nerves, indicating metabolic disorders associated with obesity and causing ER in the body (23). Findings from extensive scientific research showed that exercise exercises had beneficial effects on cognitive function and the health of the mammalian nervous system (24). Another study by Cay et al, (25) showed that endoplasmic endothelial stress markers (XBP-1, ATF6 and CHOP), which are major causes of apoptosis in the cell, are controlled by exercise activity. Swimming can reduce the levels of XBP-1, PERK and JNK proteins in obese mice (18). In a study, after 3 months of aerobic and resistive combination training, mRNA and levels of GRP78, XBP-1 and eIF2α proteins were reduced in peritoneal and peripheral adipose tissue adipose tissue (26). Endoplasmic stress has increased in relation to the expression of PERK, XBP-1s and eIF-2α genes and proteins in dystrophy and obese mice, but due to seven weeks of aerobic activity, apoptosis symptoms were not reported (27).

In some studies, the results are not consistent with the present study. For example, in a study that had been conducted in a short period of time such as one-day swimming or a 5 days activity in one week, the effect on the amount of proteins PERK, XBP-1s and eIF-2α were not significant (18), which may be due to the duration of exercise. Intense or endurance sports activities with at least 4 weeks of training can influence on the amount of these proteins. Sports can reduce endoplasmic endothelial stress, cell apoptosis, and endoplasmic endothelial inflammation in metabolic patients (28). Other studies showed that endoplasmic stress in the musculoskeletal system with its symptoms, such as GRP78, PERK, IRE1 (XBP-1) and CHOP, has increased in aerobic exercise and resistance exercise, but this increase did not result in cellular degradation and cell apoptosis. This can express the positive response of the endoplasmic endothelium to sports activities and thus lead to homeostasis in the cell (18). Interestingly, various studies have shown that mice with history of exercise activity are comparable with the mice that do not activate their symptoms of stress and the expression of UPR genes (XBP-1, ATF6 and Bip) and their CHOP after activity resistance sport was less (18).

Since exercise activity is one of the proposed therapies for improving inflammation and prevention in metabolic disorders, such as type II diabetes and insulin resistance, some studies aimed at identifying intermediate molecular mechanisms in the effect of physical activity in this study to investigate the effect exercise on XBP-1 protein and ER response. The XBP-1 protein levels reduce following aerobic exercise (20). Sport exercises have protective effects on neurons against physiological and pathological death and as a non-therapeutic intervention have a positive effect on the improvement, growth and function of the neuronal network (24). Aerobic activity has emerged as a low-cost treatment for improving neuro-cognitive function, which is more accessible to people and has unobtrusive complications that often occur with drug therapy (28).

There are significant evidences that low intensity exercise is an important factor in neurodegenerative diseases and motor function (26). In this study the non-sampling simultaneously from the tissue of all rats, absence of simultaneous measurement of all tissues, failure simultaneously measuring glucose and weight in all rats can be noted as limitations. In future studies it is suggested the evaluation of resistance training and the combination of endurance and resistance training on the amount of XBP-1 proteins in different tissues of diabetic rats and the measurement of other endoplasmic endothelial membrane proteins in diabetic rats.

Conclusions

It can be concluded that endurance sports activity in diabetic rats helped to maintain the XBP-1 protein level in its normal state and could be considered as an interventional method in diabetes and diabetes-induced neuropathy. It is suggested that this study be performed on muscles as well as patients with neuropathy.

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