ORGINAL ARTICLE

The Effect of Endurance Training Intensity on the Expression of Perlipin a Protein of Subcutaneous Adipose Tissue and Pancreatic B-cells Function in Diabetic Rats

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Abstract

Objective: The aim of this study was to compare the three endurance training intensities on the expression of Perlipin A protein in subcutaneous adipose tissue and pancreatic B-cells function in male diabetic rats.

Materials and Methods: In this study, 40 healthy male wistar rats were divided into five groups, including diabetic with low intensity endurance training, diabetic with moderate intensity endurance training, diabetic with high intensity endurance training, diabetic control and healthy control. After diabetic induction with streptozotocin, endurance training was performed with low intensity, moderate and severe for eight weeks, three sessions per week. The relative expression of Perilipin A was measured by western blot technique.

Results: The results indicated a significant effect of endurance training with three intensities on serum levels of insulin and glucose and pancreatic B-cells function (*P*-value: 0.001). Also the results showed that there was no significant difference between Perlipin A expression in healthy and diabetic control groups with endurance training groups (with low, moderate and high intensity) (*P*-value: 0.07).

Conclusion: However, Moderate and high intensity endurance training compared to low-intensity training can compensate for the loss caused by diabetes in the expression of the Perlipin A protein but the difference was not significant. It seems that more intensity endurance training lead to more increase in Perlipin A expression in diabetic rats.

Keywords: Perlipin A, Endurance training, subcutaneous adipose tissue, Diabetes, pancreatic B-cells function

Introduction

Longitudinal studies have shown that the progression of pancreatic beta cells dysfunction is very important in the prevalence of diabetes (1,2). Adipose tissue has an important role in controlling whole-body glucose homeostasis in both normal and

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disease status (3). SCAT cells may act as a buffer or sink for circulating FFAs and TGs, but once they reach their capacity they lose their protective benefit, fat begins to accumulate in tissues not suited for lipid storage (4). However, most research has pointed to the importance of visceral adipose tissue in glucose metabolism and insulin resistance (5,6,8).

In adipocytes, triglyceride is generally stored in lipid droplets (a neutral lipid core surrounded by a phospholipid monolayer coated with proteins). Positioned at the lipid droplet surface, PAT proteins manage access of other proteins (lipases) to the lipid esters within the lipid droplet core and can interact with cellular machinery important for lipid droplet biogenesis. Genetic variations in the gene for the best-characterized of the mammalian PAT proteins, perilipin, have been associated with metabolic phenotypes, including type 2 diabetes mellitus and obesity (10). Mammalian LDs contain a specific set of proteins called the PAT domain family that include perilipin, adipose differentiation-related protein (ADRP, also called adipophilin (plin2)), a tail-interacting protein of 47 kDa (TIP47) (plin3), S3-12, and OXPAT (plin5) sharing regions of conserved sequences, named PAT domains, mainly at their N termini (11). Perlipin A is one of the most abundant of these proteins, which is activated by protein kinase A, and by controlling various proteins, plays a pivotal role in regulating lipid metabolism in adipocytes (12). Destruction of Prilipin A from white adipose tissue causes adipocyte fat storage disorder by increasing basal lipolysis and reducing the lipolysis stimulated by PKA and will lead to a significant reduction in white adipose tissue (13). The role of Prilipin A in WAT is lipolysis suppression in the absence of PKA stimulation and an increase in lipolysis (about 100 times) with PKA stimulation (14). Data show that modulation of lipid droplet proteins in white adipocytes is a potential therapeutic strategy for the treatment of obesity and related disorders, such as decreasing blood glucose levels and insulin resistance (15).

Physical activity has a beneficial effect on insulin sensitivity in normal as well as insulin resistant populations. (16). In terms of pancreatic function, experiments in humans and animals have variously demonstrated that exercise improves insulin resistance, increases insulin sensitivity, increases pancreatic beta-cell mass and generally enhances beta cell function and insulin tropic action, especially in type 2 diabetes (17). Sport activity can also increase the mass of the pancreatic beta cells and generally improve and enhance the function of the beta cells of the pancreas (18). Long-term exercise is a common method used to improve health and prevent disease. While physiological compatibility with long-term exercise has been well proven in many devices, such as skeletal muscle, certain changes that occur under adipose tissue, especially subcutaneous adipose tissue, are underestimated (19). Its exact mechanism for lipolysis regulation is not completely determined (20). Many sources report enhanced stimulated lipolysis in adipocytes from endurance trained subjects that is attributed to alterations in the impolitic cascade. The purpose of this study was to compare the severity of endurance training on the expression of perlipin A protein of subcutaneous adipose tissue and pancreatic B-cells function in STZ-induced diabetic rats.

Materials and Methods

Animals

In this study, 48 eight-week-old healthy male wistar rats (250±40 gr) were used in the present study purchased from Pasture Institute (Tehran, Iran). The animals were housed two per polypropylene cages at a room temperature of 22 ℃ with 12:12h dark: light cycle (lightings were turned on at 8 PM and off at 8 AM) and 60% air humidity with free access to water and rodent pellet food. Rats were divided into the following five groups randomly: the low intensity training group (n= 8), moderate intensity training group (n= 8),

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high intensity training group (n= 8), diabetic control group (n= 8) and healthy nondiabetic sedentary group (n= 8).

Diabetes was induced by intraperitoneal injection of a single dose of streptozotocin in a fasting condition (STZ, 55 mg/ body weight; Sigma, St Louis, MO, USA) (28). To reduce the effect of streptozotocin on beta cells 15 minutes before streptozotocin injection, 270 mg / kg of nicotinamide saline mice was used (29). Rats in sham just received buffer. Two days after STZ injection blood was obtained from a small nick in the tail and measured with a glucometer (Optium Xceed). Rats with a glucose concentration exceeding 300 mg/dL were considered as a hyperglycemic state (30). From the diabetic experimental group comprising 40 rats, four rats in whom diabetes was not induced and six rats in poor health were excluded.

Wistar rats carried out eight-week treadmill training with different intensities for 30 min/training and 4 days per week for 8 weeks on a standard treadmill for small rodents. Rats were divided into groups including low intensity training group (5-8 m/min, 50-60% VO2max), moderate exercise intensity group (14-16m/min, 65-70% VO2max), and high intensity exercise group (22-25 m/min, 80% VO2max) (31). In addition, voluntary continuous exercise was induced by a 10-voltage electronic stimulator. After one week and familiarity with the laboratory environment, the rats were first introduced to the rats by running on a treadmill for a week at a speed of 10 m / min for fifteen to twenty minutes of training (32).

At the end of eight weeks of endurance training in order to rule out temporary training effects of last session, tissue sampling was conducted 48 h after last exercise bout. All rats were fasted overnight by removing food from the rat's cages 12 h prior to sacrifice. They were sacrificed for tissue collection by an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (25 mg/kg). The adipose tissue was excised, frozen in liquid nitrogen and stored frozen at -80°C until further analysis. The serum glucose levels were measured by glucometer in Germany, by measuring the tip of the tail and serum levels of insulin with a rat specific ELISA kit (Insulin rat ELISA DEV8811) manufactured by Demeditec in Germany with a sensitivity of 0.1 ng / mL and pancreatic B-cells function using the following formula was obtained (33).

FPI=Fasting Plasma Insulin (ng/ml)

FPG=Fasting Plasma Glucose (mg/dl)

HOMA-B= (20×FPI)/ (FPG- 3/5)

In order to analyses of visceral adipose tissue protein expression of Prilipin A by western blot, approximately 50 mg of each visceral adipose tissue pieces was powdered with a pestle in liquid nitrogen and lysed using a 1 mL of phosphate-buffered saline (PBS).

Statistical analysis

Data are reported as means ±standard error (SE) values. To compare groups, dependent variables were analyzed by One-way variance analysis and Kruskal–Wallis. The post hoc Games-Howell test was used to determine significant differences between groups. Statistical significance was set at *P*-value< 0.05.

Ethical considerations

This research was experimental. Animal procedures were approved by Shahrekord university ethics committee of animals (SKU: MP221/2013).

Results

The results of one-way ANOVA showed significant effect of exercise on serum levels of insulin and glucose and pancreatic B-cells function (*P*-value= 0.001) (Table 1), However, the results of the post hoc Games-Howell test showed that pancreatic B-cells function was significant different between experimental groups and healthy control group (*P*-value: 0.001).

Comparison of protein expression of perilipin A by using Kruskal-Wallis test in five groups showed no significant difference (*P*-value= 0.07).

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Table 1. Compare Serum concentration of glucose and insulin, pancreatic B-cells function and perilipin A among groups

Variable	Groups	HC	DC	DHE	DME	DLE
Glucose (mg/ dl)	Mean (±SD)	156.25 (±22.94)	557.75 (±158.84)	341.500 (±91.90)	323.13 (±48.61)	497 (±25.43)
	F	18.89				
	P-value	*0.001				
Insulin (ng / ml)	Mean (±SD)	0.49 (± 0.031)	0.19 (± 0.03)	0.12 (± 0.009)	0.14 (± 0.03)	0.17 (±0.03)
	F	211.35				
	P-value	*0.001				
pancreatic B-cells function	Mean (±SD)	0.65 (±0.01)	0.007 (±0.003)	0.007 (±0.002)	0.013 (±0.011)	0.007 (±0.001)
	F	98.74				
	P-value	*0.001				
Perilipin A (Relative density of the band)	Mean (±SD)	25600 (±11300.08)	7364.50 (± 4436.32)	14483.75± (8539.62)	10857.87 (±5858.42)	8592.45 (±5401.68)
	Chi-Square	8.49				
	Df	1.40				
	P-value	0.07				

HC: Healthy control group ؛ DC: diabetic control group؛ DLE: diabetic and low intensity training group؛ DME: diabetic and moderate intensity training group ؛ DHE: diabetic and high intensity training group

The results showed that perilipin A content in training groups was more likely to increase with increasing exercise intensity than diabetic control group however, this increase was not significant. In addition, the density of bands in different groups is shown in Figure 1.

Discussion

Changes in the expression of lipid droplet adipocyte proteins, such as prelipin A cause alter lipolysis and insulin resistance (24). The findings showed that insulin and glucose levels decreased with increasing intensity of endurance training in diabetic rats. There was no difference between the experimental groups (low, moderate and high intensity exercise) of pancreatic beta cells function and the only difference was observed between the experimental groups and the healthy control group. Reducing serum levels of insulin and glucose in diabetic groups with high intensity exercise and moderate intensity exercise

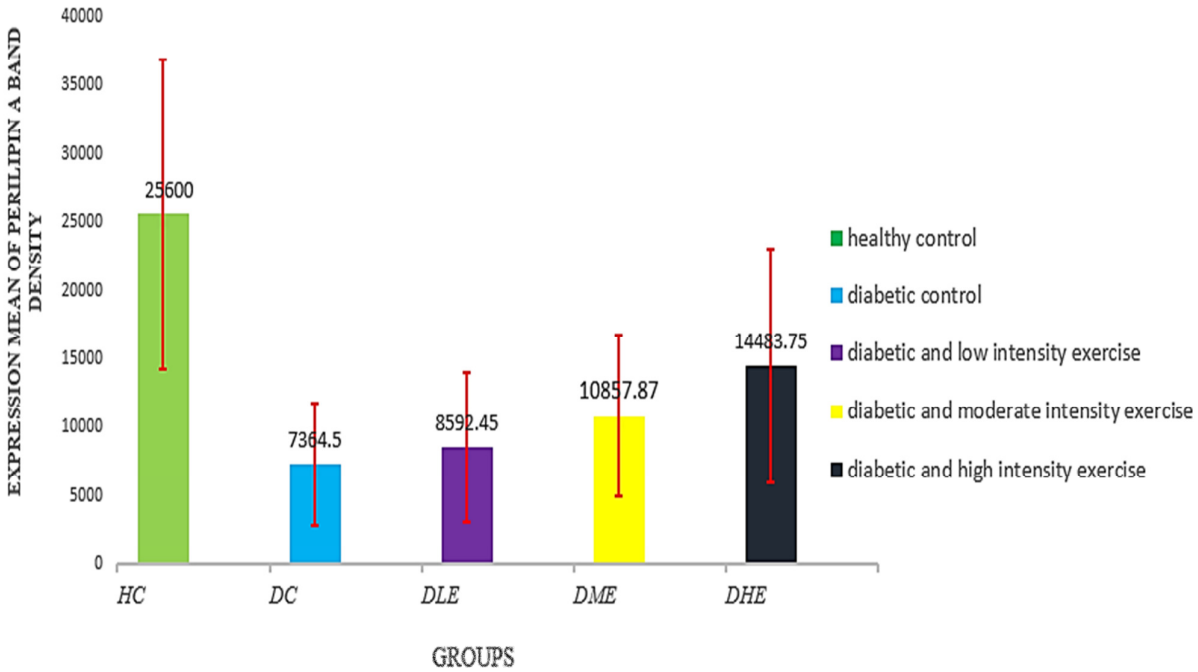

Figure 1. Changes in expression of perilipin A in groups

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compared to diabetic control and low intensity exercise was significant. Possible reducing serum insulin and glucose mechanisms through endurance exercise can include increase carrier proteins of glucose (GLUT4), decrease secretion and increase cleaning free fatty acids, increase glucose delivery to the muscles tendency to available glucose. High intensity and long duration exercise increase insulin sensitivity possibly by increasing the transfer of glucose to the muscle or reducing fatty acids synthesis and glucose reabsorption due to skeletal muscles activity (25). Also decrease in fat mass can be another reason of reducing insulin (26). Mast studies reported this decrease index following exercise schedule, have had a relatively high intensity exercise (27,28). Stephan et al. (2017) in a research showed the mean disposition index as a validated measure of β-cell function, was significantly increased after the intervention. Insulin processing inefficiency in the β -cell, expressed as the fasting proinsulin-to insulin ratio, was also reduced and increased β -cell function during the early-phase response to glucose correlated significantly with reductions in abdominal body fat (29). A recently published study of 8 weeks of high-intensity interval training, which has risen in popularity in both the exercise science field and society at large, in adults with type 2 diabetes mellitus showed improvements in β -cell function, despite no changes in insulin secretion or sensitivity (30). While Huang et al. (2011) showed that 6 weeks of voluntary exercise in type 1 diabetic rat increased the content and secretion of insulin, but did not change in the density islets of langerhans and the number of beta cells (31).

The results of some of these studies are consistent with our findings. Regarding these, the decrease in insulin and glucose levels seems reasonable in the present study. Contradiction in studies results can be caused by different factors such as nutrition type, exercise program, subject's type and intensity and duration of exercise.

Also, although there was no significant difference in the expression of perilipin A protein among diabetic training groups (low, moderate, and high). However, with increasing exercise intensity, there was a tendency to increase in perlipin A. It seems that the non-significant increase of perilipin A in diabetic rats has somewhat compensated for the reduction of perilipinA in diabetic control rat and prevents the deleterious effects of increased lipolysis and the release of fatty acids (32). Because inefficiency in lipolytic suppression and Non-esterified fatty acids (NEFA) increase when FFA demand is low, it can have serious metabolic consequences and is believed to be a key mechanism for the development of type 2 diabetes (32). Increasing the nonsignificant expression of the perilipin A in diabetic rats can also be related to the reduction of TNFα (26) and leptin (33), as well as the increase of plasma lactate (34) that may have been induced by exercise. It has been shown that TNFα reduces the expression of perlipin and increases basal lipolysis in vitro. However, the effects of TNFα on perilipinA expression should also be investigated in vivo (35). Ju Yong Bae et al in 2017 showed that after eight weeks of training, the levels of PKA, Perlipin, CGI-58, ATGL and HSL increased (36). Also, Ramos et al. (2016) showed that endurance training did not change the content of PLIN protein in epididymal white adipose tissue (37). The results of this research are consistent with the current research. Observed differences in some of this studies may be relevant to difference of type of tissue (adipose or muscle), type of fat stores (visceral or subcutaneous), type of exercise (acute or chronic), exercise protocol (duration and intensity), subjects (human and animal), type of subjects (healthy or patient).

Conclusions

Endurance training with different intensities led to changes in serum levels of insulin and glucose compared to diabetic control group. But endurance training did not significantly change the pancreatic beta cells function

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compared to diabetic control group. However, the difference was significant in comparison with the healthy control group. Also, there was no significant difference in the expression of perlipin A in the groups, but, it seems increasing intensity of endurance exercise training let to more increase in perilipin A expression in diabetic rats. It is likely that the exercise with the appropriate intensity and duration can increase the level of perlipin A, which can increase insulin sensitivity and improve insulin resistance in diabetic rats.

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