

Original Article



The relationship between the expression of PLIN3 and PLIN5 protein following endurance training in streptozotocin rats

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Abstract

Background and aims: Intermuscular lipolysis disorder plays an important role in insulin resistance and diabetes mellitus and perilipin PLIN5 and PLIN3 are the key proteins in regulating muscle cellular lipolysis. Therefore, the purpose of this study was to examine the relationship between the expression of PLIN3 and PLIN5 protein following endurance training in streptozotocin (STZ) rats.

Methods: A number of 24 male Wistar rats were randomly divided into low endurance training group (n = 8), high-intensity training group (n = 8), and control group (n = 8). Diabetes was induced in every rat by STZ injection. Three days after injection, the blood samples were taken from the cut tip of the tails of the mice and animals with blood glucose greater than 300 mg/dL were considered diabetic. The training program included eight weeks of aerobic training at different intensities. Training in high- and low-intensity groups included 22-25 and 5-8 m/min of training. Finally, one-way analysis of variance (ANOVA) and correlation was used to determine the significance of the differences between variables, followed by utilizing Tukey's post-hoc test for significance.

Results: The comparison between the groups by ANOVA showed significant differences in PLIN3 ($P=0.0006$) and PLIN5 ($P=0.012$). The results of Tukey post hoc test also demonstrated a statistical difference between the mean values of diabetic control group and high-intensity endurance group regarding PLIN3 ($P=0.01$) and PLIN5 ($P=0.009$), but no significant increase was observed in the low-intensity exercise group as compared to the control group (PLIN3, $P=0.067$ & PLIN5, $P=0.44$). As regards insulin resistance, there was a significant difference among the three groups ($P=0.0001$). Eventually, the result of the correlation between PLIN3 and PLIN5 showed similar enhancement by increasing the intensity ($P=0.0026$).

Conclusion: According to research results, high-intensity endurance training increased the expression of PLIN3 and PLIN5 in diabetic specimens and PLIN3 and PLIN5 followed a similar increase pattern in high-intensity training.

Keywords: Diabetes, PLIN5, PLIN3, Endurance Training, Intramuscular Triglycerides, Insulin Resistance

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Introduction

Diabetes is characterized by excessive fat accumulation in the form of triglycerides in non-adipose tissues such as liver, skeletal muscle, and heart (1). In addition, intramuscular triglyceride (IMTG) is associated with insulin resistance in diabetic patients (2). The concept of "lipid-induced skeletal muscle insulin resistance" demonstrates a correlation between IMTG concentrations and insulin resistance (3), as well as the relationship between IMTG and insulin resistance in individuals with low-oxidative capacity and low mobility. The low levels of IMTG are observed by increased insulin resistance as well (4). Recent evidence has shown that cytosolic regulating lipid droplet (LD) proteins play crucial roles in important cellular processes such as cellular energy homeostasis lipid storage (5). The PLIN family including PLIN1 to PLIN5 is the best-characterized family of LD proteins (6). The results of

some studies showed that the content of perilipins in type 1 muscle fibers is more than type 2 muscle fibers (7,8). Similarly, the IMTG alternate in exercise conditions is independent of the changes in PLIN protein in diabetic patients who may represent the potential role of PLIN proteins in insulin resistance. According to previous studies, PLIN3 and PLIN5 are used in intensive training and moderate-intensity exercises (8,9), while some studies indicated that the effect of training exercises on the amount of IMTG is unclear.

Although some studies reported an increase in the content of IMTG in obese patients with type 2 diabetes (10,11), other studies failed to report these changes (4,11). Furthermore, it is found that training with different intensities has different effects on the muscle LD (12,13), while Shepherd et al demonstrated that high-intensity training improves IMTG in the same way (8). Different

expressions of skeletal muscle PLIN protein are reported in the training as well.

PLIN3 and PLIN5 show engagement as the potential mediators of fatty acid or LD interaction with skeletal muscle mitochondria (14) since they are both exchangeable LD proteins that interact with intracellular organelle (14). Moreover, PLIN3 has a known role in vesicular moving in cell culture models (15) and PLIN5 is localized to mitochondria in skeletal muscle models. However, issues such as the nature of their association and what might occur under lipolytic conditions are unknown (14). PLIN5 is regarded as an LD protein, which is highly expressed in oxidative tissue (16, 17) and regulates lipid. Previous researches show that the size of LD is related to PLIN5 expression and the excess accumulation of intramuscular fat is associated with such conditions as insulin resistance and being afflicted with type 2 diabetes (18).

The intensity of physical activity is considered an important factor for diabetic patients (7). Evidence suggests that more IMTG is consumed and replenished in high-intensity interval training. Additionally, PLIN3 and PLIN5 proteins play a role in the hydrolysis of triglycerides stored in muscle LDs and the reduced turnover of IMTG is related to insulin resistance. On the other hand, considering that endurance training is able to increase IMTG, the current study sought to investigate the relationship between the expression of PLIN3 and PLIN5 proteins following endurance training in streptozotocin rats.

Materials and Methods

The current research is an experimental in vitro study. A total of 24 eight-week male Wistar rats were obtained from the Pasteur Institute Animal Care Center in Karaj, Iran. After transferring the mice to the laboratory, they were randomly divided into diabetic control, low-intensity, and high-intensity endurance training. Throughout the study, animals were maintained in normal conditions (12L/12D at 23±3°C) and fed with a standard laboratory diet, received enough water, and were housed under the same maintenance and laboratory conditions. After a few days, animals were weighed and then anesthetized with ether, and finally, received a single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg body weight. Further, 9.5 mg citrate buffer with a pH of 4.5 (sterile) was added per 1 g STZ to prepare the solution and then a yellow solution was obtained after dissolving. Prior to STZ injection, a drop of blood was taken from the cut tip of the tail of the animal in order to determine STZ injection dose. Using blood glucose (BG) test strip and BG meter, fasting blood sugar level was then measured, followed by performing the injection. Next, the blood drop was taken through the tail-tip amputation method and the blood sugar level of the animal was measured as well. Accordingly, those animals with BG greater than

300 mg/dL were considered diabetic. After one week and getting familiar with the laboratory environment, rats were randomly assigned to two training groups and one control group. Then, the rats became familiar with how to run on a treadmill for one week at a speed of 3 m/min for 15-20 minutes. The control group rats participated in no training, but in experimental groups, the training protocol was performed during eight weeks for four days (per week) for 30 minutes as follows.

- Low-intensity group. Eight rats received training at a speed of 5-8 m/min equivalent to 50%-60% Vo_{2max} .
- High-intensity group. A number of 8 rats received training at a speed of 22-25 m/min equivalent to 80% Vo_{2max} .

After performing the training protocol, all the rats were weighed 48 hours after the last training session program and then the rats were anesthetized using the intraperitoneal injections of ketamine (90 mg/kg) and xylazine (10 mg/kg). Next, the blood samples (5 cc) were directly extracted from the hearts of the mice and entered into sterile tubes, and after one hour of room temperature storage, serum isolation was conducted using centrifugation method (for 10 minutes at 2500 rpm), and the isolated serum was frozen and kept in nitrogen at -180°C. In the post-test stage, all the collected blood samples were withdrawn in one day from the refrigerator and underwent the given tests based on the related protocol. Glucose levels were measured by Germany glucometer by cutting the tip of the tail. Further, the plasma levels of insulin were estimated by ELISA kit (rhizosphere, China with 5 microns per milliliter sensitivity and the coefficient of variation of 36%). Finally, insulin resistance was calculated applying the homeostatic model assessment-insulin resistance (HOMA-IR) method by measuring insulin and fasting glucose according to the following formula (19).

$$\text{HOMA-IR} = \frac{\text{fasting insulin (ng/mL)} \times \text{fasting BG (mg/dL)}}{22.5}$$

Similarly, to analyze the soleus muscle protein expression of perilipin 5 by western blot, approximately 50 mg of each soleus skeletal muscle piece was powdered with a pestle in liquid nitrogen and lysed using a 1 mL of phosphate-buffered saline. Tissue homogenates were centrifuged at 12000 rpm for 15 minutes at 4°C and supernatant was removed as well. The total protein content of the tissue extract was determined by the Bradford method using bovine serum albumin. Accordingly, 50 µg of protein was collected per sample, separated by SDS-PAGE in 8% polyacrylamide, and electrotransferred to polyvinylidene difluoride membranes. Next, the membranes were incubated in the blocking solution (5% milk) at room temperature for 2 hours. The membranes were then incubated with primary antibodies including PLIN3 (TIP47 Antibody (B-3) 200

µg/mL, Santa Cruz) and PLIN5 (guinea pig polyclonal, Progen, #GP31, Heidelberg, Germany), and then the peroxidase-conjugated secondary antibody was directed against the primary antibody. Ultimately, the membranes were developed by an enhanced chemiluminescence western blot detection system.

Data were reported as means ± standard error (SE) values. To compare the groups, all dependent variables were analyzed by one-way ANOVA. Furthermore, Tukey post hoc test was used to determine significant differences among the groups. Data analysis was performed using SPSS software, version 21 and statistical significance was set at $P < 0.05$.

Results

At first, there was no significant difference between the weighted averages (Table 1). No significant difference was observed between the weights in the control and training groups ($P=0.47$) as well (Table 2). However, the

results of one-way ANOVA (Table 3) showed a significant difference in PLIN3 ($P=0.01$), PLIN5 ($P=0.0122$), glucose ($P=0.001$), insulin ($P=0.001$), and insulin resistance ($P=0.0001$). As shown in Table 4 and Figure 1, the results of Tukey post-hoc test also indicated that high-intensity endurance training had a significant effect on PLIN3 expression, ($P=0.01$) while no significant increase in PLIN3 was observed in low-intensity training compared to control group ($P=0.67$). Based on the results of Tukey post-hoc test, high-intensity endurance training demonstrated a significant impact on PLIN5 expression ($P=0.01$). No significant increase in PLIN5 was observed in low-intensity training when compared to the control group ($P=0.44$); the related details are presented in Table 5 and Figure 2. Table 6 shows the Tukey analysis of glucose, insulin serum levels, and insulin resistance. Table 7 and Figure 3 also represent the correlation between PLIN3 and PLIN5.

Table 1. Basic characteristics

Values	Diabetic Control Group (n=8)	Low-intensity Diabetic Group (n=8)	High-intensity Diabetic Group (n=8)
Weight before intervention (g)	201.10±14.700	271.62±24.017	182.90±17.026
Weight after intervention (g)	191.50±15.464	186.50±42.578	15.366±172.88
Glucose before interventions (mg/dL)	450.68±25.38	466.66±20.78	431.6±25.3

Table 2. The results of covariance analysis, body mass changes after eight weeks of training with different intensities

Variable	Group	Diabetic Control Group (n=8)	Low-Intensity Diabetic Group (n=8)	High-Intensity Diabetic Group (n=8)	F	P Between Groups
Weight (kg)	Pre-test	201.10±14.700	271.62±24.017	182.90±17.026	0.84	0.47
	Post-test	191.50±15.464	186.50±42.578	172.88±15.366		
	P-inside group	0.75	0.51	0.17		

Table 3. The Results of ANOVA analysis of PLIN3, PLIN5, Insulin, and HOHA-IR

Variables	Groups			ANOVA	
	High-Intensity Diabetic Group (n=8)	Low-Intensity Diabetic Group (n=8)	Diabetic Control Group (n=8)	F	P
	Mean ± SD	Mean ± SD	Mean ± SD		
PLIN3 (arbitrary)	6196±2490	4035±2402	3400.56±2497.21	5.54	0.0006
PLIN5 (arbitrary)	7294.61±1283.85	5292.22±2362.30	3400.56±2497.21	5.54	0.012*
Glucose (mg/dL)	341.50±91.905	0.1213±0.009	557.75±158.847	18.892	0.001*
Insulin	0.1213±0.009	0.1725±0.0310	0.1925±0.036	211.35	0.001*
HOMA-IR	1.823±0.4415	3.808±0.6883	4.833±1.227	25.84	0.0001*

ANOVA: Analysis of variance; HOMA-IR: The homeostatic model assessment-insulin resistance; *Significant at $P \leq 0.05$.

Table 4. The results of Tukey post hoc test on the expression of PLIN3

Group	Group	Different Mean	P
High-intensity diabetic group (n=8)	Diabetic control group (n=8)	992.5	0.01*
	Low-intensity diabetic group (n=8)	2157	0.22
Low-intensity diabetic group (n=8)	Diabetic control group (n=8)	1326	0.67

*Significant at $P \leq 0.05$.

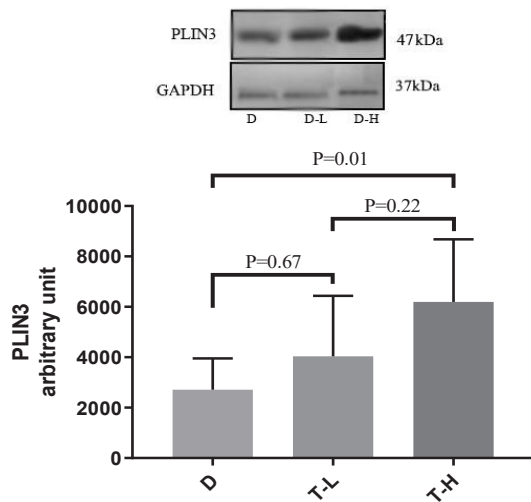


Figure 1. The effect of training on the expression of PLIN3 in soleus muscle. Note. D: Diabetic control; T-L: Low-intensity exercise; T-H: High-intensity exercise.

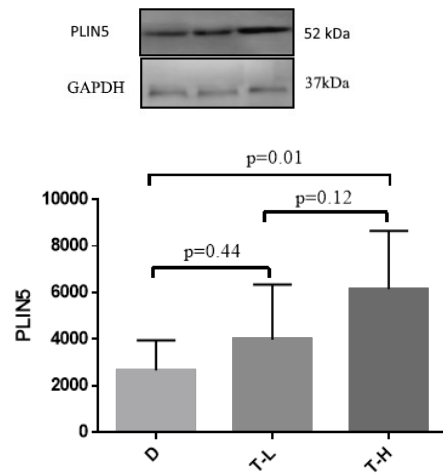


Figure 1. The effect of training on the expression of PLIN5 in soleus muscle. Note. D: Diabetic control; T-L: Low-intensity exercise; T-H: High-intensity exercise.

Discussion

Based on the results of this study, high-intensity exercise significantly increased the expression of PLIN3 and PLIN5 compared to the diabetic control group and PLIN protein increasing by high-intensity endurance training as well. STZ-induced diabetes causes muscle atrophy due to the increased levels of glucose and a decrease in insulin levels (20,21). Moreover, muscle atrophy reduces protein synthesis and increases protein degradation in skeletal muscle (22). It seems that high-intensity training has a stimulatory effect to compensate for PLIN3 and PLIN5 protein synthesis (23). Minnaard et al also highlighted the benefits of training on PLIN5 protein for the muscle rate (24). The increased PLIN3 and PLIN5 expressions were reported in animal (14) and human (25) studies after training. It was reported that PLIN5 mitochondria increase after 30 minutes of high-intensity contraction, as well as fatty acid (FA) transport and metabolism in the muscle tissue (14). Similarly, Mason et al observed the accumulation of PLIN5 and PLIN3 protein content in Vastus lateralis muscle after 60 minutes of high-intensity training (26). Another study also showed an increase

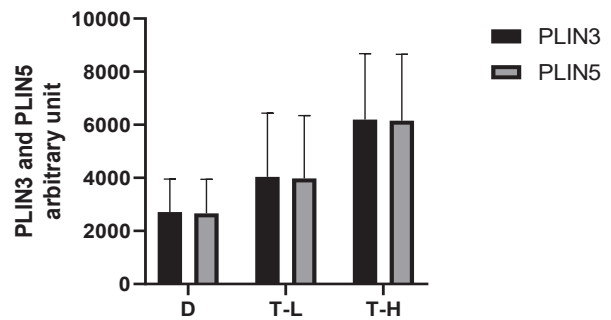


Figure 3. The relationship between PLIN3 and PLIN5 in different intensity. Note. D: Diabetic control; T-L: Low-intensity exercise; T-H: High-intensity exercise.

in PLIN5 in diabetic patients after 6 months of high-endurance training (27).

Cell culture studies demonstrate that PLIN3 and PLIN5 expression recruits adipose triglyceride lipase (ATGL) and its coactivator, as the comparative gene identification-58 (CGI-58), to the LD surface under basal conditions. It is believed that PLIN5 phosphorylation releases CGI-58 to bind ATGL to stimulate lipolysis in response to

Table 5. The results of Tukey post hoc test on the expression of PLIN5

Group	Group	Different Mean	P
High-intensity diabetic group (n=8)	Diabetic control group (n=8)	3492.85	0.01*
	Low-intensity diabetic group (n=8)	2175.71	0.12
Low-intensity diabetic group (n=8)	Diabetic control group (n=8)	1317.142	0.44

*Significant at $P \leq 0.05$.

Table 6. The results of Tukey post hoc analysis of serum insulin and glucose levels

Group	Group	Insulin		Glucose	
		Different Mean	Significant	Different Mean	Significant
High-intensity diabetic group (n=8)	Diabetic control group (n=8)	0.0712	0.000*	236.25	0.001*
	Low-intensity diabetic group (n=8)	0.0512	0.011*	155.5	0.046*

Table 7. Correlation Between PLIN3 and PLIN5

Pearson r	PLIN5 T-H vs. PLIN3 T-H	PLIN5 T-L vs. PLIN3 T-L	Total PLIN3 vs. PLIN5
R	0.99	0.99	1.000
R-squared	0.99	0.99	1.000
P value	0.0001	0.0001	0.0026

T-L: Low-intensity exercise; T-H: High-intensity exercise.

protein kinase A activation (18). Both PLIN3 and PLIN5 co-precipitated with CGI-58 at rest and following the contraction. MacPherson et al found that PLIN3 and PLIN5 proteins work together to regulate lipolysis (23). It seems PLIN5 regulates oxidative LD hydrolysis and controls the local FA flux to protect mitochondria against excessive exposure to FA during physiological stress, and therefore, reduces the insulin resistance (28).

The results of the present study revealed that low-intensity endurance training led to an increase in the expression of PLIN3 and PLIN5, but this increase was not significant while in the study by Mason et al, a significant increase was detected in endurance training with 60% Vo_{2max} (27). In contrast, no significant increase was observed in PLIN3 and PLIN5 by low-intensity interval training, and it is assumed that a limited number of muscle fibers are used by this training due to less consumption of IMTG (27).

The comparison between endurance training exercises and high-intensity interval training showed that the latter leads to more accumulation and greater fragmentation of IMTG. It also increases the expression of PLIN3 and PLIN5 and probably maintains a low concentration of muscle FA metabolites, leading to improved insulin sensitivity in endurance training (28). Our findings further indicated that insulin resistance, along with low-intensity and high-intensity endurance activities in diabetic rats, decreased significantly. Similarly, the destruction of pancreas by STZ leads to a sharp decrease in insulin levels, and owing to hyperglycemia (20), the loss of muscle mass is observed in the models of a severe decrease in insulin (30). Frequent impulses during training are also shown to coordinate the enzymes associated with the metabolism of IMTG (31), because of which endurance-trained athletes have high amounts of IMTG but are insulin sensitive. Further, the increased synthesis rates of IMTG were associated with decreased ceramide and diacylglycerol concentration (14). These data suggest that IMTG may protect against insulin resistance during increased free FA uptake (32).

Conclusion

In general, the obtained data demonstrated that the levels of PLIN3 and PLIN5 increase in response to high-intensity endurance training and thus decrease insulin resistance. Furthermore, the expression of the skeletal muscle PLIN5 protein relies on the intensity of training and both of these proteins have almost an ascending trend.

Conflict of interests

None.

Ethical considerations

All rules and procedures for working on animals (i.e., getting to know, training, anesthesia, and animal killing) were according to AAALAC (Association for Assessment and Accreditation Laboratory Animal Care International) and under the Code of Ethics Number 2398727IRSKU in Shahrekord University.

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