The effect of interval training intensity on protein levels of ATGL and Perilipin 5 in visceral adipose tissue of type 2 diabetic male rats

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ABSTRACT

In adipose tissue, adipose triglyceride lipase (ATGL) and perilipin5 (PLIN5) play an important role in the regulation of lipolysis. However, little is known about the effect of interval training intensity on ATGL and PLIN5 in visceral adipose tissue of type 2 diabetic male rats. In this study, forty male Wistar rats were assigned into two groups as follows: healthy control (HC) (n=8) and T2D (n=32) groups which were fed with their corresponding diets for 10 weeks. After the induction of diabetes, CD animals and 8 rats from the D group were sacrificed (first experiment), and the remaining T2D animals were divided to moderate intensity interval training (MIIT), high intensity interval training (HIIT) and control (DC2) groups. Interval training (HIIT, MIIT) was applied for 12 weeks, 5 sessions per week. The MIIT protocol included 13 bouts of 4-minute activity with equivalent intensity of 60-65% vo2max and the HIIT protocol included 10 bouts of 4-minute activity with equivalent intensity of 85-90% vo2max with 2-minute active rest periods. The Western Blot method was used to measure PLIN5 and ATGL protein levels. ANOVA and Tukey's test were used for data analysis. The results indicated that type 2 diabetes resulted in a significant increase in the protein levels of PLIN5 (p<0.01) and non-significant increase in the protein levels of ATGL (p=0.19). In contrast, both HIIT and MIIT protocols led to a significant decrease in protein levels of PLIN5 (p= 0.001), while they had no significant effects on protein levels of ATGL (p>0.05). When compared to the CD2, there was no significant difference between the two training groups in protein levels of PLIN5 and ATGL (p>0.05). Our finding indicated that interval training, independent of the exercise intensity, resulted in suppression of diabetes-induced PLIN5 which may be involved in the stimulation of visceral adipose tissue lipolysis.

KEY WORDS: Type 2 diabetes, Interval Training Intensity, lipolysis, PLIN5 and ATGL,

INTRODUCTION

Obesity is considered as an important risk factor for type2 diabetes [1, 2], by increasing the levels of triglyceride and lipoproteins with low density and lowering the lipoproteins with high density. Diabetes type 2 is characterized by fat accumulation in non-fatty tissues such as liver, skeletal muscle and heart [3]. Increased lipolysis and fat oxidation, especially visceral fat, due to exercise training can be an important therapeutic goal for type 2 diabetic patients [4]. The concentration of free
plasma fatty acids (FFA) is the result of a balance between lipolysis products and fat oxidation by muscle, heart, liver and other tissues. Thus, impaired lipolysis regulation can significantly affect plasma FFA levels. Indeed, a decrease in lipolytic activity may lead to the accumulation of adipose tissue, and an increase in lipolytic activity along with inhibition of insulin mediated metabolism, may increase circulating FFA concentrations [5]. Plasma FFA mainly inhibits glucose utilization by affecting muscle tissue. Therefore, excessive fat oxidation in the skeletal muscle leads to a decrease of insulin-stimulated glucose uptake. In fact, FFA metabolism is associated with glucose uptake in patients with type 2 diabetes mellitus [6] and regulation of lipolysis in adipose tissue may play an important role in these patients.

Perilipins (PLINs) are proteins that are located on the surface of lipid droplets and contribute to lipolysis through regulation of the HSL by phosphorylation and dephosphorylation [7]. There are complex and numerous variations of covering proteins of fat droplets whose any type plays an important role. Some of them are located on fat droplets, while others are located on fat droplets, depending on the conditions which include lipogenic and lipolytic enzymes. However, the function of PLINs is less known [8]. Recent studies show that a number of proteins covering GTPase have been identified that are involved in the transference of lipase adipose tissue (ATGL) to fatty droplets, especially fatty droplets covered by PLIN5 [9]. Recently, PLIN5 has been considered, due to its dual subcellular status and its activity through exercise training. PLIN5 can be expressed in both fat droplets and mitochondria and involved in the interactions between fat droplets and mitochondria. The content of mitochondrial PLIN5 increases with muscle contraction, which indicates the role of PLIN5 in the regulation of substrate oxidation [9]. PLIN5 is a target for peroxisome proliferator-activated receptor gamma (PPARγ) and induces the transcription of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) activated by exercise. Increasing the expression of PLIN5 results in an enhancement of fat droplets in the skeletal muscle cells, increased expression of genes involved in oxidative metabolism and increased brown adipose tissue, as well as an increase in the expression of target genes for PPARγ [10]. However, the regulatory role of lipolysis for PLIN5 is still unclear. Some studies confirmed the facilitator role and some others the inhibitory role for PLIN5 in lipolysis [10].

In addition to the activity of PLIN5 for lipolysis of adipose tissue, another important lipase has been identified that plays a key role in lipolysis of lipid droplet [11]. ATGL has a potent glycerol hydrolyzate triglyceride activity [12] whose catalytic activity increases by co-activating protein such as CGI-58 and inhibited by G0S2. It is now clear that ATGL acts as a mediator for beta-adrenergic induced lipolysis and TG in adipose tissue cells, as the removal of ATGL causes obesity and expressing a high level of ATGL produces a slimming phenotype [13]. Additionally, the inhibition of ATGL in human adipose tissue reduces the total TG lipase activity to 80%, which implies the important role of ATGL in lipolysis of human adipose tissue [14].

Regular exercise is an important strategy for the treatment of many metabolic disorders, including type 2 diabetes and obesity. Part of the beneficial effects of exercise training is through the regulation of fat mass, especially visceral fat, which plays an important role in regulating energy metabolism and insulin resistance [15]. In addition to reducing the size of adipocytes, increasing the enzymes involved in mitochondrial biogenesis [16, 17] and increasing the expression of metabolic cytokines [18], exercise training can reduce lipid content in adipose tissue, although effective molecular mechanisms affecting this adaptation are less well-known. It has been well established that exercise training can lead to the stimulation of adipose tissue lipolysis in a pathway dependent on beta-adrenergic receptors and cAMP [19]. However there are very limited studies regarding the effects of exercise training on the levels of adipose tissue PLINs and ATGL as new effective makers and regulators of lipolysis. Previous studies have identified interactions between PLIN3 and PLIN5 with ATGL, HSL and CGI-58 in resting state without alteration after this contraction [20, 21]. Moreover, in skeletal muscle, PLIN5 muscle protein content increases after endurance training and sprint training [14, 22]. Regarding the changes in adipose tissue PLINs to exercise training, it has been recently reported that PLIN5 was the only protein from the PLIN family that responded to endurance training in the subcutaneous fat tissue of women with polycystic ovary syndrome [23]. What remains unknown is that the changes in the PLIN5 and ATGL protein content of visceral adipose tissue are as a new marker of lipolysis regulator to exercise training.

In recent years, high intensity interval training (HIIT) has been identified as an effective exercise intervention that can have similar or greater benefits from moderate to moderate intensity training (MICT) [24]. For example, HIIT interventions have been shown to have similar effects to MICT in metabolic adaptation, cardiovascular fitness, and body composition, and more beneficial effects to improve glycemic control. In addition, more effects of HIIT on triglyceride hydrolysis during exercise and more storage of triglycerides after exercise have been reported [25]. However, the effect of interval training intensity on the response of visceral adipose tissue ATGL, PLIN5 is not known.
Given the importance of studying novel mediators of lipolysis and limited studies on the effects of exercise training on these markers, in the current study first we investigated the effect of type 2 diabetes on visceral adipose ATGL and PLIN5. Then, in order to investigate the effect of exercise intensity, we compared the effect of high and moderate-intensity interval training on visceral adipose tissue ATGL and PLIN5.

METHODS

All animal experiments were conducted according to the National Institute of Health ethical guidelines for the care and use of laboratory animals (NIH; Publication No. 85-23, revised 1985) and were reviewed and verified by the Veterinary Ethic Committee of Guilan University of Medical Sciences (Approval ID: IR.GUMS.REC.1397.044). The method used in this study was experimental with post-test design with control group. For this purpose, 40 8-week-old male Wistar rats with a weight range of 180 ± 20 g were purchased from Pasteur Institute of Iran. After 2 weeks of adaptation, rats were randomly divided into two groups: healthy control (HC) (n=8) and DT2 (n=32) groups. Animals were fed a CD or HFD ad libitum for 10 weeks. After 10 weeks of consuming high-fat diet, diabetes was administered with streptozotocin single-injection (STZ) solution in sodium citrate buffer with Ph=4.5 up to 30 mg/kg with intraperitoneal (IP) method. Diabetes was verified 96 hours later by evaluating blood glucose levels with the use of glucose-oxidase reagent strips (Glucometer 01, Japan). The rats having blood glucose level of 300 mg/dl (11.1 mM) or greater were considered to be diabetic. Afterwards, HC animals and 8 rats from the DT2 group were sacrificed to determine the effect of DT2 on visceral adipose tissue ATGL and PLIN5 (First experiment). Subsequently, the remaining DT2 animals (n=24) were randomly divided into three subgroups as follows: High intensity interval training (HIIT) (n=8), moderate intensity interval training (MIIT) (n=8) and diabetic control group (DC2) (n=8). Animals in the HIIT and MIIT groups completed their exercise training protocol for 12 weeks/5 sessions a week, while DC2 group did not receive any exercise program during these 12 weeks. The HC and DT2 groups after induction of type 2 diabetes and HIIT, MICT and DC2 groups after 12 weeks of exercise protocols, after an overnight fasting, were anesthetized with ketamine (60 mg/kg) and xylazine (6 mg/kg) injection and blood samples were collected from animals following overnight fasting through cardiac puncture. Blood samples were centrifuged at 12000 g for 10 min and serum were separated and kept at -80 °C before analysis. In addition, mesenteric visceral adipose tissues were rapidly isolated and then frozen in liquid nitrogen and kept at -80 °C before analysis.

Training protocols

The HIIT protocol was performed for 12 weeks and 5 sessions per week on a treadmill as previously described, which included 10 bouts of 4-minute high intensity running with 85-90% VO2max and 2 minute active rest periods with 50% VO2max. The interval pace was increased gradually over 10 weeks and maintained for the next two weeks (eleventh and twelfth weeks). Accordingly, the treadmill speed was increased gradually from 25 m/min in the first week, reached 34 m/min in the tenth week and maintained at this value for the last two weeks. The MIIT protocol was performed for 12 weeks and 5 sessions per week on a treadmill where the covered distance was matched to that of HIIT protocol, which included 13 bouts of 4 min moderate intensity running with 65-70% VO2max and 2 minute active rest periods with 50% VO2max. The interval pace was increased gradually over 10-week and maintained for the next two weeks (eleventh and twelfth weeks). Accordingly, the treadmill speed was increased gradually from 16 m/min in the first week, reached 25 m/min in the tenth week and maintained at this value for the last two weeks [47].

Serum Analysis. Enzyme-linked immunosorbsent assay (ELISA) kits (MyBioSource) were used to measure serum insulin (MBS724709) levels. Glucose levels were evaluated by the glucose oxidase method. In addition, insulin resistance index was assessed by homeostasis model assessment (HOMA-IR) and calculated using the formula: HOMA-IR= [fasting concentrations of glucose (mmol/L) × insulin (mU/L)] /22.5.

Western blotting.

Protein lysates from cells and tissues were isolated using lysis buffer (50mM Tris, pH 7.5, 150mM sodium chloride, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.1mM EDTA and 0.1mM EGTA) supplemented with complete protease inhibitor cocktail (Roche, Germany). Protein contents were determined by the Bradford method. Proteins were separated using SDS– polyacrylamide gel electrophoresis using 8–12% denatured ready gel (Bio-Rad, Hercules, CA, USA) and transferred on to a PVDF membrane. The membrane was blocked for 1 h in 5% BSA in Tris-Buffered Saline and 0.1% Tween 20 (TBST) to block nonspecific bindings. Subsequently, blots were incubated overnight at 4 °C with primary antibodies (purchased from Santa Cruz Biotechnology) and secondary goat anti-rabbit IgG-HRP. Incubation in secondary goat antirabbit IgG-HRP (sc-2004, dilution 1:1,000), goat anti-mouse (sc-2005, dilution 1: 1,000) was performed the next day, for 1 h at room temperature in 5% milk in TBST. Protein bands were visualized with an enhanced chemiluminescence (ECL) reagent and quantified by densitometric analysis with Image J software.

Statistical analysis.

A one-way ANOVA followed by Tukey post-hoc tests was performed for comparison between groups. All data were represented as the mean±SEM. P-values <0.05 were considered to be statistically significant.
STATISTICAL RESULTS

Body weight, insulin resistance index, and serum levels of glucose, insulin. We found a significant difference in the serum levels of glucose (F(4, 25) = 60.18, P=0.001) and insulin (F(4, 25) = 28.61, P=0.001) and insulin resistance index (F(4, 25) = 0.61, P=0.002) in study groups. Our results demonstrated that the induction of type 2 diabetes significantly increased body weight (F(1, 38) = 58.48, P=0.001) and serum levels of glucose (P<0.001) and decreased insulin resistance index and serum levels of insulin (P<0.05 for both), compared to the CD group (Table 1).

In contrast, both HIIT and MICT protocols significantly decreased serum levels of glucose (F(P<0.01 for both), but did not significantly effect on insulin resistance index (P>0.05 for both) and insulin serum levels (P>0.05 for both). Also, there was no significant difference between the research groups after performing of exercise protocols in body weight (F(2, 21) = 2.61, P=0.09) (Table 1).

Visceral adipose tissue ATGL and PLIN5. In order to evaluate the effect of type 2 diabetes and exercise training on protein levels of ATGL and PLIN5 in the visceral adipose tissue, these proteins levels were measured by Western blotting. We found a significant difference in the PLIN5 (F(4, 25) = 26.48, P=0.001), and non-significant difference in the protein levels of ATGL (F(4, 25) = 2.04, P=0.19) in study groups. The findings revealed that type 2 diabetes resulted in a significant increase in the protein levels of PLIN5 (P<0.001), and non-significant increase in the protein levels of ATGL (P>0.05), when compared to the HC group.

In contrast, both HIIT and MICT protocols markedly reduced protein level of PLIN5 (P<0.001 for both), when compared to the CD1. However, there was no significant difference between the two training groups (P>0.05). Moreover, analysis showed that both HIIT and MICT protocols resulted in no significant differences in the protein levels of ATGL (P>0.05 for both), when compared to the CD2 (Figure1).

Table 1. Effect of type 2 diabetes and exercise training on body weight and metabolic factors

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (gr)</th>
<th>Serum glucose (mg/dl)</th>
<th>Serum Insulin (ng/ml)</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>274.00±12.86</td>
<td>117.37±1.93</td>
<td>1.65±0.25</td>
<td>0.47±0.06</td>
</tr>
<tr>
<td>CD1</td>
<td>339.12±13.07*</td>
<td>280.83±24.19*</td>
<td>0.50±0.08*</td>
<td>0.34±0.03*</td>
</tr>
<tr>
<td>CD2</td>
<td>342.50±36.99</td>
<td>243.66±29.79</td>
<td>0.62±0.13</td>
<td>0.37±0.04</td>
</tr>
<tr>
<td>HIIT</td>
<td>381.75±53.19*</td>
<td>183.16±16.94*</td>
<td>0.71±0.26</td>
<td>0.31±0.10</td>
</tr>
<tr>
<td>MICT</td>
<td>359.75±36.28</td>
<td>187.33±13.79*</td>
<td>0.63±0.21</td>
<td>0.28±0.08</td>
</tr>
</tbody>
</table>

HC: health control (standard diet), DC1: diabetes control1 (firs experiment), DC2: diabetes control2, MICT: moderate intensity interval training. HIIT: high intensity interval training. * Data are represented as means ± SEM (n=6): *p<0.05..
DISCUSSION

This study investigated the exercise training intensity effect on visceral adipose tissue ATGL, PLIN5 and insulin resistance in diabetic male rats. The results showed that type 2 diabetes and exercise training have different effects on the ATGL, PLIN5 protein levels. Our major findings showed that both exercise trainings (HIIT and MIIT) resulted in a decrease of protein levels of visceral adipose tissue PLIN5, but ATGL changes in response to exercise training (HIIT and MIIT) were not significant. Nevertheless, the induction of type 2 diabetes resulted in a significant increase of protein levels of visceral adipose tissue PLIN5 and a slight increase of visceral adipose tissue ATGL. Moreover, both exercise trainings (HIIT and MIIT) resulted in improvement of serum glucose, while, they had no significant effects on insulin resistance and serum insulin.

The beneficial effects of exercise training on diabetes type 2 and insulin resistance-associated diseases are well-known, which may be due to the regulation of adipose tissue lipolysis. HSL and ATGL are the key enzymes involved in lipolysis and PLIN5 proteins have interfered in the regulating lipolysis by interacting with HSL and ATGL [26]. However, the effects of exercise training on these lipolysis regulators are less well-known. The major finding of current study showed that exercise training, independent of exercise intensity, resulted in an increase in PLIN5 in visceral adipose tissue. Previous studies have shown that LIN5 is an important regulator of substrate metabolism in sedentary mice [27-29] as new studies provided evidence that PLIN5 is dispensable for substrate metabolism during exercise [29]. Moreover, In line with current observation, it has been reported previously that PLIN5 is required for the metabolic adaptations and enhancement in exercise tolerance following endurance exercise training [29]. This is likely to be an important adaptation, because a PLIN5 coats the surface of intracellular lipid droplets, where it promotes TAG storage by inhibiting lipolysis under metabolic and hormonal conditions of energy sufficiency. The exact mechanism of inhibition remains unclear, but a prevailing notion is that cytoplasmic PLIN5 binds either ATGL or CGI-58, an activator of ATGL lipase activity, and thereby prevents their interaction with each other [30]. In addition, the findings of the present study indicate that there is no difference between HIIT and MIIT in the expression of PLIN5 protein levels. This study, for the first time, investigate the effect of the exercise training intensity on the protein levels of PLIN5 in adipose tissue. Previously available studies in this regard, have been reported similar findings from the effects of SIT and MICT on the expression of PLIN5 in lean individuals [14] and obese males [31] in skeletal muscle. In fact, the reduction of PLIN5 with interval training may lead to the stimulation of visceral lipolysis through to the removal of the inhibitory role of this protein from lipolysis and subsequently and the reduction of this tissue which is one of the main causes of insulin resistance in type 2 diabetes. However, the link of PLIN5 with fat oxidative capacity may have a dual origin, PLIN5 may be involved in the release of fatty acids from the LD as ligands for PPAR mediated gene expression and hence facilitate induction of oxidative genes, with increased fat oxidative capacity as a consequence [32]. On the other hand, via promoting the interaction of ATGL and CGI-58 on the LD, PLIN5 supposedly controls lipid droplet lipolysis [33], possibly with the aim to tune lipolytic rate to the rate of fat oxidation [34]. More importantly, compared to type 2 diabetes patients (T2DM), trained subjects have high levels of PLIN5. On the other hand, endurance training up-regulate PLIN5 protein content in skeletal muscle and Intramyocellular lipids (IMCL) hamper insulin sensitivity albeit not in endurance-trained athletes (Trained) [35]. However, it has been reported that Abundance of PLIN5 in skeletal muscle of trained athletes relative to patients with type 2 diabetes cannot explain the athlete’s paradox in a direct and straightforward manner [35]. The findings of the current study showed that induction of type 2 diabetes leads to an increase of PLIN5 protein levels. In this regard, there is no similar study to evaluate the effect of diabetes on visceral PLIN5. Nevertheless, Covington and et al (2015) reported that the expressions of PLIN3, along with PLIN5, is greatly reduced in adipose tissue of women with PCOS when compared to age-and body composition-and metabolically-matched females [23]. While, it has already been reported that high-fat feeding up-regulates PLIN5 at both mRNA and protein levels [36]. LD play a critical role in oxidative tissues to maintain appropriate fuel supply during periods of energy needs but also to buffer daily fluxes of FA to avoid cellular lipotoxicity. PLIN5 has been previously shown as a LD protein inhibiting lipolysis and correlating with insulin sensitivity [37, 38]. In fact, according to a recent study, PLIN5 protects against palmitate-induced insulin resistance and facilitates FA oxidation in response to muscle contraction and increased metabolic demand in vitro [36]. However, the present study suggests that induction of type 2 diabetes leads to the up-regulation of visceral adipose tissue PLIN5, and interval training resulted in suppression of PLIN5 protein expression.
Lipolysis in white adipocytes is regulated by a multifaceted phenomenon that is subject primarily to distinct temporal controls such as hormonal stimulation via catecholamines. The hormonal activation of lipolysis in adipocytes is mediated via a traditional cAMP-dependent signal transduction process. An increased intracellular cAMP level phosphorylates and activates cAMP-dependent protein kinase A (PKA) and subsequently phosphorylates hormone-sensitive lipase (HSL) [39]. In addition to HSL, ATGL is thought to be the first and rate-limiting step in lipolysis. It is responsible for catalyzing the reaction of removing the first fatty acid from a triglyceride molecule, releasing the fatty acid for various metabolic fates, and subsequently, producing a diglyceride [40]. Exercise training in this cohort increased adipose tissue lipolysis under adrenergic stimulation [41]. Recent findings have been identified that ATGL protein increased in all three skeletal muscles in response to 8 weeks endurance training. Yet the largest relative increase is in glycolytic gastrocnemius, which typically relies the least on fat as a fuel source [42]. In addition, other studies also reported that ATGL protein content was increased in the trained group compared with the sedentary group in skeletal muscle [43, 44]. To date, however, little is known about the effect of exercise training on the molecular changes of ATGL in white adipose tissue. In the present study we observed that interval training resulted in only a very slight increase in the content of visceral adipose tissue ATGL. Protein levels and mRNA of ATGL, and HSL proteins all are up regulated by exercise training and that PPARγ2 are closely associated with the exercise training-induced up regulation of ATGL [45]. In fact, up regulation of PPARg-2 would have the capacity to modulate protein synthesis of ATGL. In contrast, insulin induced down-regulation of ATGL via transcription factors such as FoxO1 which reported transrepresses PPARg target genes via direct protein-protein interaction, and insulin induces FoxO1 phosphorylation and nuclear exportation, which prevents FoxO1-PPARg interactions and results in the rescue of the FoxO1-induced transrepression of PPARg [46]. Also, Insulin attenuates intracellular cAMP production through increases in phosphodiesterase-3B (PDE-3B) activity, which changes cAMP to AMP via the activation of protein kinase B/AKT [39] accordingly, because of the decrease in insulin as a result of type 2 diabetes, non-significant increase of ATGL may be due to the reduction of this inhibitory role of insulin on this enzyme. In contrast, both HIIT and MIIT did not have a significant effect on insulin, and were associated with the slight increase of insulin. Therefore, lack of ATGL response to interval training can be due to lack of insulin changes as a result of exercise training, which were independent of the intensity of exercise, because there was no significant difference between the two groups in the insulin response. More importantly, the phosphorylation of lipases plays a central role in the regulation of enzyme activity and is closely associated with the catabolism of adipocytes. Two phosphorylation sites of ATGL, at Ser404 and Ser428, have been identified in the C-terminal region in humans [40], which regulates ATGL activity. In the present study, there is no data about phosphorylation of ATGL content as a limitation of this study. As a result, interval training may be associated with stimulation of visceral lipolysis in interaction with PLIN5 by increasing the activity of ATGL through phosphorylation of this enzyme.

This study has generated data that exercise training decreased protein levels of PLIN5 in visceral adipose tissue which was increased with diabetes. These results indicate that interval training (HIIT and MIIT) can lead to the activation of visceral fat lipolysis, which is independent of the exercise intensity, due to increased protein levels of PLIN5 and stimulation of ATGL activity.

The current study is limited by deficiency measurement of visceral adipose tissue mass and protein levels of other Perilipin family (including Perilipin 2 and 3) as well as the assessment of whole-body insulin sensitivity by OGTT. Therefore, further work is required to determine the role of Perilipin family proteins in the regulation of adipose tissue lipolysis and improving insulin sensitivity by adaptation to exercise training.

CONFLICT OF INTERESTS STATEMENTS
The authors declare no conflict of interest.

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