High-intensity interval training increases mitochondria biogenesis in adipose tissue and improves insulin resistance in high fat diet-induced obese rat

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ABSTRACT

The purpose of this study was to investigate the effects of high intensity interval training (HIIT) on PGC-1α and mtDNA of subcutaneous adipose tissue and insulin resistance in obese male rats. After inducing obesity with high fat diet (for 10 weeks), eight rats from the high-fat diet group (O) and eight rats of the standard dietary group (C) were sacrificed and other obese rats were randomly divided into two groups: obesity control (OC, n=8) and high intensity interval training (HIIT, n=8). The HIIT protocol includes 10 bouts of 4-minute activity with equivalent intensity of 85-90% vo2max and 2 minute active rest periods for 12 weeks and 5 sessions per week. Western Blot method was used to measure PGC-1α and real time PCR was used to measure. Obesity induced by a high-fat diet resulted in a significant reduction in protein levels of PGC-1α [0.25±0.13 vs. 1] and insignificant reduction in gene expression of mtDNA [0.80±0.02 vs. 1]. In contrast, HIIT resulted in a significant increase in protein levels of PGC-1α [1.64±0.40 vs. 0.29±0.03] and gene expression of mtDNA [4.92±0.59 vs. 0.89±0.02]. In addition to, HIIT resulted in a significant decrease in serum levels of insulin [1.49±0.15 vs. 2.14±0.43 ng/ml] and glucose [111.16±8.07 vs. 154.66±13.21 mg/dl] and improved insulin resistance insulin [1.96±0.20 vs. 3.89±0.54]. It seems likely that HIIT result in increase of adipose tissue mitochondrial biogenesis in obesity which may be involved in improving insulin resistance.

KEY WORDS : High intensity interval training, mitochondrial biogenesis, insulin resistance, obesity

INTRODUCTION

Obesity has become a global healthcare problem as a consequence of imbalance between energy intake and consumption which it's associated metabolic disorder including insulin resistance. Adipose tissue is one of the largest organs in the body and functions in lipid storage, and energy homeostasis [1]. Mitochondrial oxidative phosphorylation and substrate oxidation represent the main energy source for cell [2]. In adipose tissue, mitochondrial function or dysfunction plays an important role in energy homeostasis [3] and reduced mitochondria number and/or function is associated with obesity [4, 5]. In particular, mitochondrial disorders have been reported to play a role in the pathogenesis of obesity, insulin resistance, and progression of type 2 diabetes [6]. The role of exercise in the treatment and prevention of metabolic diseases, such as obesity, has been well documented [7]. Exercise training result in adaptation in various tissues of the body, especially the adipose tissue. In adipose tissue, exercise training reduce adipocytes size and fat content and enhance the enzyme involved in fat oxidation [8]. While the molecular mechanisms responsible for the beneficial effects of
exercise training less well-known, exercise training improves mitochondria in both skeletal muscle and adipose tissue [9, 10], which may play an important role in reducing lipid accumulation, oxidative stress, and insulin resistance. In support of this hypothesis, the content of mitochondria [skeletal muscle and adipose tissue] is reduced in the insulin resistance model [11], while treatment regimens that increase mitochondrial density are associated with improved insulin sensitivity [12].

PGC-1α, one of the most important transcription factors, regulates the genes involved in both adipogenic and mitochondrial biogenesis by stimulating PPAR-γ. PGC-1α in addition to stimulating the oxidation of fatty acids by increasing the PPARα activity [13] and browning the white adipose tissue through UCP-1 stimulation [14], is responsible for mitochondrial biogenesis by activating NRF1/2 nucleus and increasing expression of mtTFA [15, 16]. In fact, PGC-1α is recognized as the most important metabolic regulator for mitochondria biogenesis, which may be a therapeutic target for metabolic disorders.

Previous studies have reported that exercise training can have an effective role in up-regulation of PGC-1α and mitochondrial biogenesis by activating intracellular signaling, such as AMPK, p38MAPK and CaMK [17]. Although important factors in regulating intracellular signaling and mitochondria biogenesis by exercise training are involved, it has been suggested that exercise intensity is a key factor in mitochondrial function and remodeling. In skeletal muscle, studies have shown that exercise increase PGC-1α protein in a pattern dependent on exercise intensity [18-20]. In this regard, high intensity interval training (HIIT) is known to be a potent activator for mitochondrial biogenesis in skeletal muscle, which can be induced by the expression of PGC-1α [18, 19, 21]. For example, Little and et al [2010] demonstrates that a practical model of low volume HIT is a potent stimulus for increasing skeletal muscle mitochondrial capacity [22]. Also, it has been reported that acute bout of low-volume HIT activates mitochondrial biogenesis in skeletal muscle through a mechanism involving increased nuclear abundance of PGC-1α [18].

Furthermore, similar findings have been reported on the role of HIIT in diabetic subject. Such that, Little and et al [2011] demonstrates that HIT reduced hyperglycemia and increased muscle mitochondrial capacity in patients with type 2 diabetes [23]. However, in white adipose tissue, the PGC-1α and mitochondria biogenesis response to HIIT remains unknown. Therefore, in this study, we first investigate the effect of high fat diet-induced obesity on mitochondria biogenesis markers of subcutaneous adipose tissue [PGC-1α and mtDNA]. Then we investigate the effect of HIIT on PGC-1α and mtDNA and insulin resistance in high fat diet-induced obese rats.

METHODS

Thirty two male adult Wistar rats, seven-week-old, were purchased. After a week of adaptation, in a temperature-controlled room and maintained with food and drink ad libitum in a 12:12 h light-dark cycle and 25±2 °C temperature, animals were randomly divided into two groups: control diet (CD) (n=8) and HFD (n=24) groups. Animals were fed a CD (contained 10% fat, 70% carbohydrates, and 20% protein) or HFD (contained 60% fat, 20% carbohydrates, and 20% protein) ad libitum for 10-wk period [24]. After 10 weeks, rats of CD and eight rats of HFD groups were sacrificed to determine the effect of induction of obesity by high-fat diet (First experiment). Then, other HFD fed rats were divided into the following subgroups: HFD+HIIT and HFD+SED. After completing the training protocol, rats of HFD+SED and HFD+HIIT groups were sacrificed to determine the effect of exercise training in high-fat diet obesity (second experiment). At the end of both experiments, after an overnight fasting, rats were anesthetized with Ketamin (6.6mg/kg) and Xylazine (0.3mg/kg) intra-peritoneally and blood samples and subcutaneous adipose tissue (groin) were rapidly isolated and then were frozen in liquid nitrogen and stored at ~80 °C.

Training protocols. HFD+HIIT completed the HIIT protocols for 5 days/week for 12 weeks in conformity with a protocol modified from that of Hafstad et al [2013,2011] [25, 26]. The HIIT protocol included 10 bouts of 4 min high intensity running with 85-90% VO2max and 2 minute active rest periods with 50% VO2max. The interval pace was increased gradually over 10-week and maintained for the last two weeks (eleventh and twelfth Weeks) [25, 26]. Accordingly, the treadmill speed was increased gradually from 17 m/min in the first week, reached 26 m/min in the tenth week and maintained at this value for the last two weeks. Vo2max was measured using a treadmill (25° inclination) to control and adjust the intensity of exercise. The speed was gradually increased until exhaustion off despite increased running speed, where Vo2max was defined. The running speed at which Vo2max was obtained was defined as speedmax [25, 26].

**Serum Analysis.** Serum insulin levels were measured by Enzyme-linked immunosorbsent assay (ELISA) kits (MyBioSource) (MBS724709). Homeostatic model assessment insulin resistance (HOMA-IR) was determined according to the following equation.

\[
\text{HOMA-IR} = \left( \frac{\text{fasting concentrations of glucose (mg/dl)} \times \text{insulin (\muU/L)}}{2430} \right)
\]

**Western blotting.** Western blot analysis were then performed as previously described [28], using the following primary antibodies: PGC1α [SC5815] and GAPDH (6C5) (sc-32233) (both from Santa Cruz). Protein bands were visualized with an enhanced chemiluminescence (ECL) reagent and quantified by densitometric analysis with Image J software. Protein levels were normalised by the formula that the band density of the target protein was divided by that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the same sample and are reported as fold values of the levels obtained for the control group.
Real-time PCR analysis. The ratio of mitochondrial DNA (mtDNA): nuclear DNA (nDNA) were measured to determine the relative number of mitochondria in the subcutaneous adipose tissue. Total cellular DNA from white adipose tissues was extracted using the DNeasy blood and tissue kit and the relative levels of mtDNA and nDNA were quantified using primers specific for mitochondrial Cox2 (mt-Cox2; forward, 5'-GCCGACTAATAA CAAGCAACA-3'; and reverse, 5'-CAATGGGCTAAAGCTATGG-3') and the nuclear gene Hbb-b1 (forward, 5'-GAAGCGATTCTAGGGAGCA G-3'; and reverse, 5'-GGAGCA GCGAGATCTGAGTAG-3').

Statistical analysis. All data are presented as mean±SD. one-way ANOVA followed by Tukey post-hoc tests was used to determine significant differences among groups. P-values<0.05 were considered to be statistically significant. All statistical analyses were performed through the use of a statistical software package (SPSS, Version 20.0).

Ethics statement. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the Veterinary Ethics Committee of Guilan University of Medical Sciences (Approval ID: IR.GUMS.REC.1397.081).

STATISTICAL RESULTS
In the first experiment, we found a significant difference between study groups in insulin (F (4,25) = 7.39, P=0.001), glucose (F (4,25) = 14.65, P=0.001), and insulin resistance index (F (4,25) = 14.51, P=0.001). Our results demonstrated that 10-week HFD significantly increased insulin resistance index [4.14±0.47 vs. 2.30±0.13] (p<0.01), serum levels of insulin [2.37±0.16 vs. 1.65±0.10 ng/ml] (p<0.01) and glucose [146.16±9.92 vs. 117.37±0.79 mg/dl] (p<0.01) compared to the CD group. In contrast, HIIT significantly decreased serum levels of insulin [1.49±0.15 vs. 2.14±0.43 ng/ml] (p<0.01) and glucose [111.16±4.07 vs. 154.66±13.21 mg/dl] (p<0.001) and insulin resistance index [1.96±0.20 vs. 3.89±0.54] (p<0.01) (Table1).

Moreover, in order to evaluate the effect of obesity and HIIT on the mitochondria biogenesis markers, protein levels of PGC-1α and gene expression of mtDNA were measured by Western blotting and RT-PCR. Our results showed that a significant difference between study groups in protein levels of PGC-1α (F (4,25) = 4.67, P=0.001), and gene expression of mtDNA (F (4,25) = 207.93, P=0.001). HFD significantly down-regulated PGC-1α protein levels [0.25±0.13 vs. 1] (p<0.001) and insignificant reduction in gene expression of mtDNA [0.80±0.02 vs. 1] (p>0.05) compared to the CD group. Moreover, HIIT significantly increased protein levels of PGC-1α [1.64±0.40 vs. 0.29±0.03] and gene expression of mtDNA [4.92±0.59 vs. 0.89±0.02] compared to the HFD+SED group (p<0.001, for both) (Figure1).

Table 1. Effect of high-fat diet and exercise training on 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>CD</td>
<td>274.00±12.80</td>
<td>117.37±1.93</td>
<td>1.65±0.25</td>
<td>2.87±0.40</td>
</tr>
<tr>
<td>HFD</td>
<td>366.62±24.02**</td>
<td>146.16 ± 24.3**</td>
<td>2.37 ± 0.36**</td>
<td>5.17±1.44*</td>
</tr>
<tr>
<td>HFD+SED</td>
<td>428.87±21.88</td>
<td>154.66±13.21</td>
<td>2.14±0.43</td>
<td>4.86±0.68</td>
</tr>
<tr>
<td>HFD+HIIT</td>
<td>407.87±21.97</td>
<td>111.16 ± 4.07***</td>
<td>1.14 ± 0.15**</td>
<td>2.45±0.25**</td>
</tr>
</tbody>
</table>

[CD: control diet; HFD: high-fat diet; HFD + SED: high-fat diet + sedentary; HFD + HIIT: high-fat diet + high intensity interval training (HIIT). * Data are represented as means ± SEM (n=6): *p<0.05. ** Data are represented as means ± SEM (n=6): *p<0.01. *** Data are represented as means ± SEM (n=6): *p<0.001]
DISCUSSION

Obesity is related with important metabolic disorders, including the development of insulin resistance and type 2 diabetes. In contrast, the beneficial effects of exercise training on obesity and obesity-related diseases are well known, which may be due to biogenesis and mitochondrial function. Our major findings at the first intervention showed that high-fat diet induced obesity resulted in a significant reduction in the protein level of PGC-1α, as well as an insignificant reduction in the mitochondrial contents of subcutaneous adipose tissue. In contrast, HIIT resulted in a significant increase in mitochondrial contents and protein levels of PGC-1α. Many previous studies have reported that exercise training may be leads to increase mitochondrial biogenesis and its content in skeletal muscle, but there are very limited studies about the effects of exercise training on this pathway in adipose tissue.

PGC-1α, as a transcriptional co-activator, is the main regulator that stimulates the browning of white adipose tissue and the mitochondria biogenesis in white adipocytes [29]. In fact, PGC-1α regulates the activity of NRF1/2 (transcription factors) which, by controlling the expression of TFAM (key protein of mitochondrial transcription), accelerates the proliferation and expression of mtDNA gene [15]. Based on the results of this study, the severe reduction of PGC-1α and a slight decrease in mtDNA after high-fat diet induced obesity in subcutaneous adipose tissues indicated the regulatory role of PGC-1α in obesity-induced insulin resistance. In support of the findings of this study, previous studies have shown that obesity with a high-fat diet in rats causes glucose homeostasis disorder, which was related with reduction of PGC-1α expression and mtDNA content in adipose tissue [30]. Exercise training provide an acceptable strategy for stimulating the expression of PGC-1α in obese samples, while it seems that HIIT is a potent activator for expressing PGCI-α in adipose tissue previously reported in skeletal muscle [18, 19]. Multiple complex networks of intracellular signaling pathways and hormones play a role in mitochondrial biogenesis. AMPK and SIRT1 are known as two upstream regulators for PGC-1α signaling, which are linked to the mitochondria biogenesis [31]. The findings of previous studies indicate that the expression of the SIRT1 mRNA and the activity of AMPK in adipose tissue are significantly reduced by moderate and severe obesity [32]. In contrast, HIIT lead to phosphorylation of AMPK, which was associated with an increase in PGC-1α expression [33]. Additionally, an increase in PGC-1α expression following up-regulation of SIRT1 after 12 weeks of exercise training has also been reported [34]. Therefore, according to the different responses of AMPK and SIRT1 [upstream regulators of PGC-1α] to obesity and exercise training, reduction of PGC-1α as well as mtDNA consequently inducing obesity may be due to decreased activity of the AMPK / SIRT1 pathway. On the contrary, increasing PGC-1α and mtDNA in response to HIIT may be due to increased SIRT1 and AMPK phosphorylation. In agreement with the upstream role of AMPK and SIRT1 in regulating PGC-1α in skeletal muscle, recent evidence suggests that AMPK [35] and SIRT1 [36] play an important role in activating PGC-1α in adipose tissue. AMPK is regulated through the levels of ADF and SIRT1 through cellular NAD+ levels [35, 36], which are two intracellular metabolic sensors, and their expression and activities increases with depletion of training-dependent intracellular energy storage. In fact, AMPK via phosphorylation and SIRT1 through deacylation results in activation of PGC-1α. Additionally, AMPK can up-regulate NAD metabolism and SIRT1 activity to increase PGC1-α activation [37]. Based on the findings of the present study, HIIT has a significant effect on the expression of mtDNA and PGC-1α, possibly due to decrease in intracellular energy in a pattern dependent on exercise.
intensity. As previously reported, exercise an intensity-dependent pattern results in AMPK phosphorylation [38], and other studies reported a similar pattern for expression of PGC-1α in skeletal muscle, indicating a higher stimulation of PGC-1α in response to HIIT [18, 19].

In addition, the tumor suppressor protein, p53, has recently been recognized as a regulator of mitochondrial function, with PGC-1α having a P53-linked site in its promoter region [39]. Whole-body knockout (KO) of p53 in mice has low levels of PGC-1α protein and mitochondria in the skeletal muscle [39]. In contrast, acute contractile activity has been shown to increase P53 phosphorylation by increasing AMPK and p38MAPK [33, 39]. However, it has recently been reported that P53 activity is dependent on the intensity of exercise, so that only the sprint interval training (SIT) results in an increase in P53 [20]. This increase in P53 is known as a marker for intensity dependent PGC-1α activity [20]. Therefore, increased expression of PGC-1α and mtDNA may be related to the activity of P53 as a result of HIIT. The present study provided for the first time evidence that HIIT can stimulate the mitochondrial biogenesis of subcutaneous adipose tissue in obese rats.

High-fat diet induced obesity resulted in increased serum glucose and insulin and increased insulin resistance. Obesity is characterized by the accumulation of fat in the form of triglycerides and the increase in the size of adipocytes that contributes to the development of insulin resistance. Previous studies reported that the growth and enlargement of adipocytes is an independent marker of insulin resistance [40]. Moreover, the increase of fat storage in other tissues such as the liver and skeletal muscle due to saturation of adipocytes from fat may attribute to insulin resistance [41]. In the current study, the body weight of HFD-fed rats continually increased during the procedure, and HFD group gained significantly more body weight than CD group. Therefore, according to the findings of the present study, obesity with high-fat diet led to an increase in insulin resistance which may be due to the deployment of inflammatory status and hypertrophy of adipocytes. In contrast the reduction of adipocytes hypertrophy could improve insulin resistance. Exercise is considered as an important strategy for weight loss and reduction of size of adipocyte [42], and so is the potential mechanism for insulin resistance by increasing energy expenditure. In line with the findings, many previous studies have been supporting the improvement of insulin resistance in response to HIIT [43]. Therefore, in present study, HIIT may improve insulin resistance by reducing the size of adipocytes and improving the inflammation status. Consistent with this hypothesis, fat mass loss and reducing the size of adipocytes size have been reported after eight weeks of HIIT in obese fatty rats that related to improved insulin resistance [44]. Additionally, improvement in mitochondrial function as well as mitochondria biogenesis through HIIT may be another reason for improving insulin resistance. As previous studies in this area have reported the reduction of PGC-1α is a reason for the development of insulin resistance [45]. Therefore, increasing the expression of PGC-1α and mitochondria content by HIIT-depend pathway may lead to improved insulin resistance in obese rats.

Overall, the most important finding of the present study for the first time showed that HIIT result in an increase in the expression of mtDNA and protein levels of PGC-1α in subcutaneous adipose tissue, which were down-regulated by high-fat diet induced obesity.

The most important limitation of the present study was the lack of measurement of fat mass, protein levels of NRF1 and TFAM as well as signaling of AMPK and SIRT. Therefore, further work is required to determine role of upstream signaling pathway on mitochondria.

CONFLICT OF INTERESTS STATEMENTS

The authors declare no conflict of interest.

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