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Research paper

Comparative studies on the effects of high-fat diet, endurance training and obesity on *Ucp1* expression in male C57BL/6 mice

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ABSTRACT

Background: Obesity triggers a variety of severe conditions, therefore deteriorates metabolism rate of adipose tissues and muscles. Uncoupling proteins which are highly stimulated by fatty acids are potential targets for anti-obesity agents through breaking the electron gradient in the mitochondrial matrix and creating imbalances in the electron transport chain, thereby increasing the amount of substrate used to produce energy. Therefore, the aim of present study is assessment of exercise and high fat diet on expression level of *Ucp1* subcutaneous white and brown adipose tissues (scWAT & BAT) respectively.

Methods: To perform experiments, 48 male C57BL/6 mice were divided to two major groups and fed with high fat diet (HFD) or low fat diet (LFD) during a period of 12 weeks. After the first intervention, each groups was divided into four groups randomly as (HF-EX), (HF-SED), (LF-EX), (LF-SED) [EX: exercise; SED: sedentary] in form of treadmill running for 45 min/day, 5 days/week during 8 weeks. One day after the last practice session, mice were sacrificed and *Ucp1* expression was assessed on scWAT & BAT.

Results: Data indicated a down-regulation in scWAT *Ucp1* in obese mice similar to what observed for the expression of *Pgc1α*. Both, BAT *Ucp1* and *Pgc1α* mRNA decreased significantly in response to obesity and physical activity. Moreover, exercise caused significant decrease in scWAT mitochondrial proteins contradictory to BAT.

Conclusion: Taken together, exercise exerted controversial effects compared with HFD and obesity on expression of *Ucp1* and *Pgc1α* in scWAT dissimilar to BAT tissues, concluding that obesity may cause a resistance to exercise in terms of metabolic demands for scWAT tissue.

1. Introduction

Obesity is an epidemic global problem related to metabolic syndrome and type-2 diabetes (T2D) (Varela-Rodriguez et al., 2016; Joffin et al., 2015; Moreno et al., 2015). This severity may be related to adipose and muscles tissues, the main metabolic centers in body (Tsiloulis and Watt, 2015; Lo and Sun, 2013). Uncoupling proteins (UCPs) serve as potential targets for anti-obesity agents. UCP1 is mainly distributed in brown adipose tissue (BAT) while it has been detected in specific white adipose depots (Castrejon-Tellez et al., 2016; Busiello et al., 2015; Irving et al., 2014). The free fatty acids released from the triglycerides stored in the body are the precursor in activation of UCP1

Abbreviations: BAT, brown adipose tissue; FFA, free fatty acids; Gapdh, Glyceraldehyde 3-phopahte dehydrogenase; ROS, reactive oxygen species; RT-qPCR, real-time quantitative PCR; scWAT, subcutaneous white adipose tissue; SNS, sympathetic nervous system; T2D, type 2 diabetes; UCP, uncoupling protein; visWAT, visceral white adipose tissue

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(Harms and Seale, 2013; Nedergaard et al., 2001). The main outcome of UCP functions is decrease in ATP production resulting in regulation of fuel metabolism. Additionally, UCP1 interfere in free fatty acids (FFA) oxidation which ultimately prevents progress of obesity and T2D (Zhang et al., 2014; Knaub et al., 2013).

Subcutaneous white adipose tissue (scWAT) has been found to express higher amount of UCP1 compare to visceral white adipose tissue (visWAT). An increase in WAT *Ucp*1 transcripts may account as converting of WAT to BAT with a heat-generating property (Tsiloulis and Watt, 2015; Lo and Sun, 2013; de Queiroz et al., 2012; Ringholm et al., 2013; Rocha-Rodrigues et al., 2016; Morton et al., 2016). Exercise (EX) effect on adipose thermogenesis is already unraveled by β3-adrenergic receptors stimulation with sympathetic nervous system which is generally high in exercise. Hence, exercise is the effective way to control obesity and T2D throughout an increase in UCP1 content and raising metabolism in scWAT and turn it to beige adipose tissue (Ohyama et al., 2015; Wu et al., 2012; Dhamrait et al., 2012).

Considering the obesity, as a global problem and UCP1 action as one the most nominated factor in this field which is regulated by *Pgc1α* this study designed to evaluate whether how endurance training combined with diet could serve as a deteriorating factor in obesity to affect the expression of *Pgc1α*, *Ucp1* and mitochondrial content in scWAT/BAT to explore a non-pharmaceutical intervention in regulation of energy balance in human.

2. Materials and methods

2.1. Animals

Forty-eight male C57BL/6 mice (Pasteur Institute, Tehran, Iran), 6–8 weeks age, weighing about 12–16 g, were kept in normal conditions (12-h light/12-h dark cycles, temperature of about 23 ± 1 °C, and humidity of 50–60%). The animals had ad libitum access to water and pellet rodent diet. All experiments were performed in accordance with the international guide for the care and use of laboratory animals and the experimental procedures were evaluated and approved by the Ethics Committee of Royan Institute.

2.2. Diet

After a habitation week, mice were randomly divided into two groups, low fat diet (LF) (10% fat, 20% protein, 70% carbohydrate) and high fat diet (HF) (45% fat, 20% protein, 35% carbohydrate). This condition continued for 12 weeks and mice named as obese and non-obese groups. Then in the second phase of study each two groups divided in to four groups, 1) HF/EX, 2) LF/EX, 3) HF/SED, 4)LF/SED accordingly (Fig. 1).

2.3. Exercise protocol

Training mice started the exercise protocol at the 7 m/min for 15 min per day on a treadmill, and within 2 weeks, the activity gradually increased to 45 min per day and the intensity of the activity went-up to 17 m per minute. The two primary weeks were designed to allow mice to start on the treadmill for 45 min a day at a speed of 17 m/min. From this step onwards, according to an increasing practice protocol, the mice trained on a treadmill each week for 5 consecutive days each day for one session and 45 min for each session. In the first two weeks, with intensity 17 m/min, the second the weeks 19 m/min, fifth and sixth weeks 21 m/min, and two final weeks 23 m/min. Control groups that were not physically trained, were placed on a treadmill for 8 weeks, 5 days a week and 15 min each session, so that all conditions, including manipulation of the mice by the investigator, were the same for all groups, and the only difference between experimental and control groups was in exercise program.

2.4. Tissue sample collection

Twenty four hours after the last training session, the mice were sacrificed with 14 h of fasting. Inguinal WAT as scWAT, visWAT, muscle and *interscapular BAT, were* obtained. Weight of tissues was measured and snap-frozen in liquid nitrogen, and stored at −70 °C for gene expression analysis.

2.5. RNA isolation and gene expression

Tissues were processed according to the Trizol protocol for extraction of RNA (Thermo Fisher Scientific Ambion Trizol LS Reagent), overnight −70 °C incubation in isopropanol phase. Following the dissolution of RNA, cDNA synthesis followed immediately with Thermo Fisher Scientific kit (RevertAid First Strand cDNA Synthesis Kits, Thermo Fisher Scientific, USA). Real-time quantitative PCR (RT-qPCR) was performed with SYBR green fluorescent dye using a Corbett Rotor-Gene 6000 Real Time PCR System. Data were assessed and reported according to the ΔΔCt method. The list of primers is available in supplementary table 1.

2.6. Statistical analysis

To describe the data, standard error of mean and central indexes were used. To examine the effect of training on dependent variables, two way ANOVA test used. Data were analyzed using SPSS software (version 18). The significance level was considered at $p < 0.05$. Also, Pearson correlation analysis was performed between variables.

3. Results

3.1. Body weight, calorie consumption and adipose tissue weight

As illustrated in Fig. 1A, primary 12 weeks of first intervention included weight gaining process, and the rest of study contained 8 weeks of grouping and the start of second intervention. Weight gaining and calorie consumption of mice during 20 weeks of investigation are demonstrated and were correlated ($p < 0.05$) (Fig. 1A and B for obese mice and Fig. 1C and D for non-obese mice). Furthermore the effect of high fat diet in the process of obesity and weigh gaining was significant (Fig. 2) ($p < 0.05$). Supplementary table 2 shows the effect of HFD and exercise on body weight and WAT to body weight. Also, the ratio of BAT to body weight rose in response to HFD, obesity and exercise ($p < 0.05$). Supplementary table 3 demonstrates the effect of HFD, obesity and exercises on mentioned factors and represents HFD and exercise exerts a significant effect on WAT weight. BAT weight modulated significantly in response to all diet and exercise while muscle remained unchanged ($p < 0.05$).

3.2. Gene expression regulation

Glyceraldehyde 3-phopahte dehydrogenase (Gapdh) expression was used to normalize transcripts of all genes in different tissues and as examined, there were no intervention-induced differences in *Gapdh* (data not shown).

3.3. Analysis and expression levels of Pgc1α in scWAT and BAT

Modulations in *Pgc1α* expression of scWAT and BAT with different interventions are represented in Fig. 3A and C respectively. As indicated, *Pgc1α* mRNA levels were modulated significantly in response to HFD and obesity in scWAT (Fig. 3B) ($p < 0.05$). Furthermore, BAT *Pgc1α* transcripts modulated significantly under obesity and exercise conditions (Fig. 3D) ($p < 0.05$).

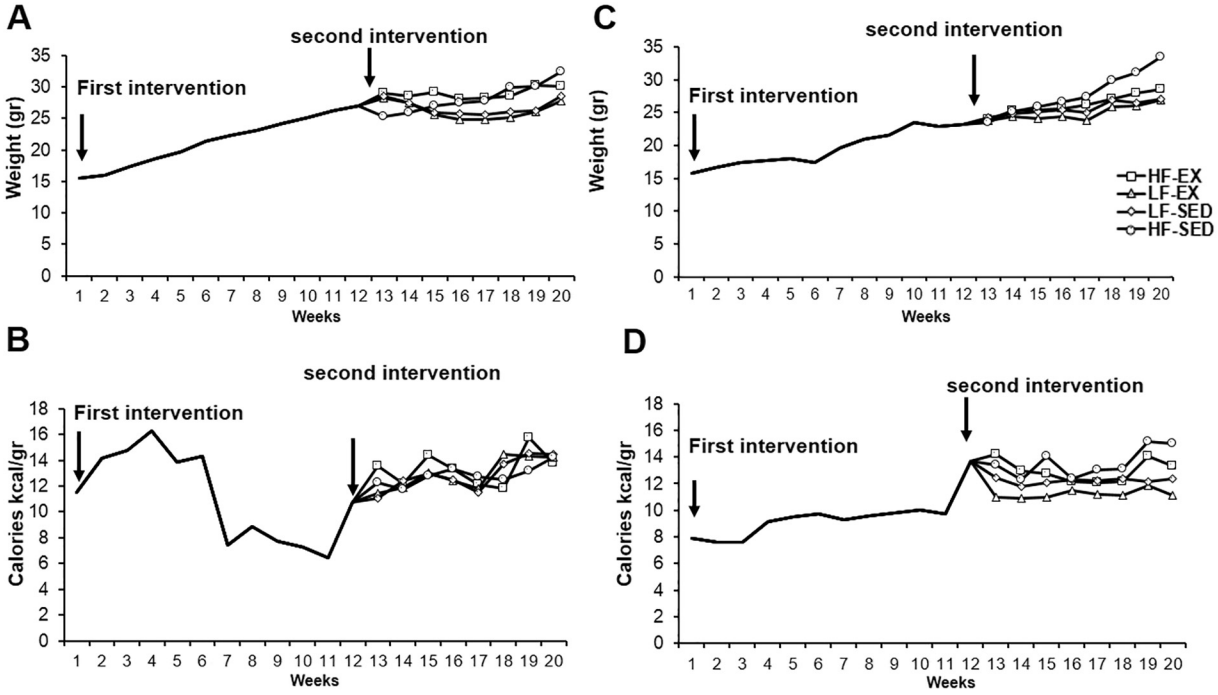

Fig. 1. Calorie consumption and weight gaining of obese and non-obese mice.
Assessments of Calorie consumption and weight gaining of obese and non-obese mice during two interventions (first intervention [12 weeks] and second intervention [8 weeks]) are shown. During the first intervention mice were divided in two groups and received either HFD or LFD. At the second intervention, the mice in each group were divided randomly into 4 groups (in each group, n = 6) (HFD/sedentary, HFD/exercise, LFD/sedentary, LFD/exercise) as described in materials and methods. Weight gain and calorie consumption of obese (A and B) and non-obese (C and D) mice are indicated. Data were evident that the rate of calorie consumption was increased in obese mice. Arrows indicate the starting of intervention sessions.

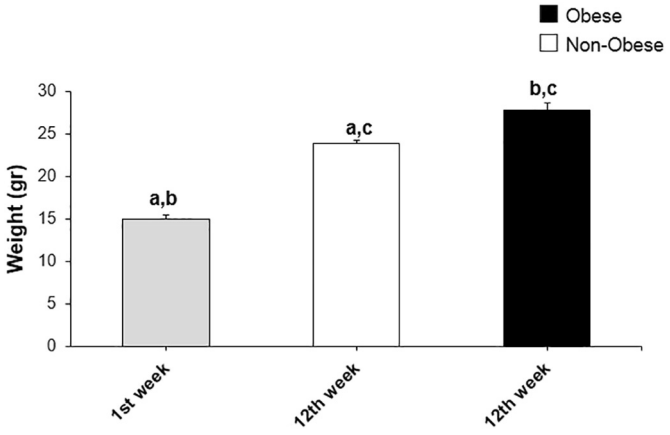

Figure 2. Effect of HFD on weight gain.
First column shows the average weight of mice in the first week. The weight shows a significant change in obese groups vs. non-obese groups ($p < 0.05$). Similar alphabets indicate significant change between the same samples (each group, n = 24).

3.4. Analysis and expression levels of Ucp1 in scWAT and BAT

Fig. 4A and C shows scWAT and BAT *Ucp1* mRNA levels respectively whereas Fig. 4B and D indicates the effect of interventions in aforementioned tissues respectively. Notably, scWAT *Ucp1* mRNA levels rose significantly in response to exercise while it was reduced under HFD ($p < 0.05$) (Fig. 4B). Meanwhile, BAT *Ucp1* mRNA up-regulated in response to HFD whereas, obesity and exercise, down-regulated this parameter (Fig. 4D) ($p < 0.05$). Of note, correlation analysis between *Pgc1α* and *Ucp1*expression in BAT indicated a significant correlation ($p < 0.01$) between these two parameters (Supplementary Fig. 1).

3.5. Analysis and mitochondrial protein content of scWAT and BAT

Mitochondrial deposits of scWAT and BAT (Fig. 5A, C), in different groups of mice respectively. To explain the effect of interventions, data were rearranged as shown in Fig. 5B and D. Of note, a promotion in scWAT mitochondrial content was acquired in response to HFD and obesity dissimilar to exercise ($p < 0.05$) (Fig. 5B). Inversely, exercise raised the amount of mitochondrial protein content of BAT ($p < 0.05$) (Fig. 5D).

4. Discussion

Preservation of an appropriate body weight through diet and exercise training is a key factor and therefore obesity is considered as a major health hazard (Joffe and Houghton, 2016). In term of obesity control, it remains dubious what molecular pathways may change the progression of WAT by differential expression of UCPs and change it to beige adipose tissue to combat obesity (Linden et al., 2014).

Obesity, such as what is clarified in previous studies, caused a reduction in *Pgc1α* mRNA in BAT and scWAT (Moreno-Navarrete et al., 2013; Moreno-Santos et al., 2016). Also obesity results in reduction of *Ucp1* in both scWAT and BAT. This could be due to the signaling pathways which disturb obesity in body and healthy metabolic profile changes during adaptations (Dantas et al., 2017; Bargut et al., 2016). Previous studies stated that *Pgc1α* is one of the major regulators of *Ucp1*. Hence any change in *Pgc1α* expression results in similar trend on *Ucp1* expression (Ringholm et al., 2013; Betz et al., 2012). Likewise, obesity leads to impaired metabolic pathways, so called “leptin resistance”. Leptin is considered to be one of major contributor of *Ucp1* in BAT and despite an increase in leptin amount, receptors in the hypothalamus would become insensitive. In order to this phenomenon, neuronal signals to BAT decrease and therefore *Ucp1* would not be able to uncouple the respiration in long time (Tsukita et al., 2012; Ueta

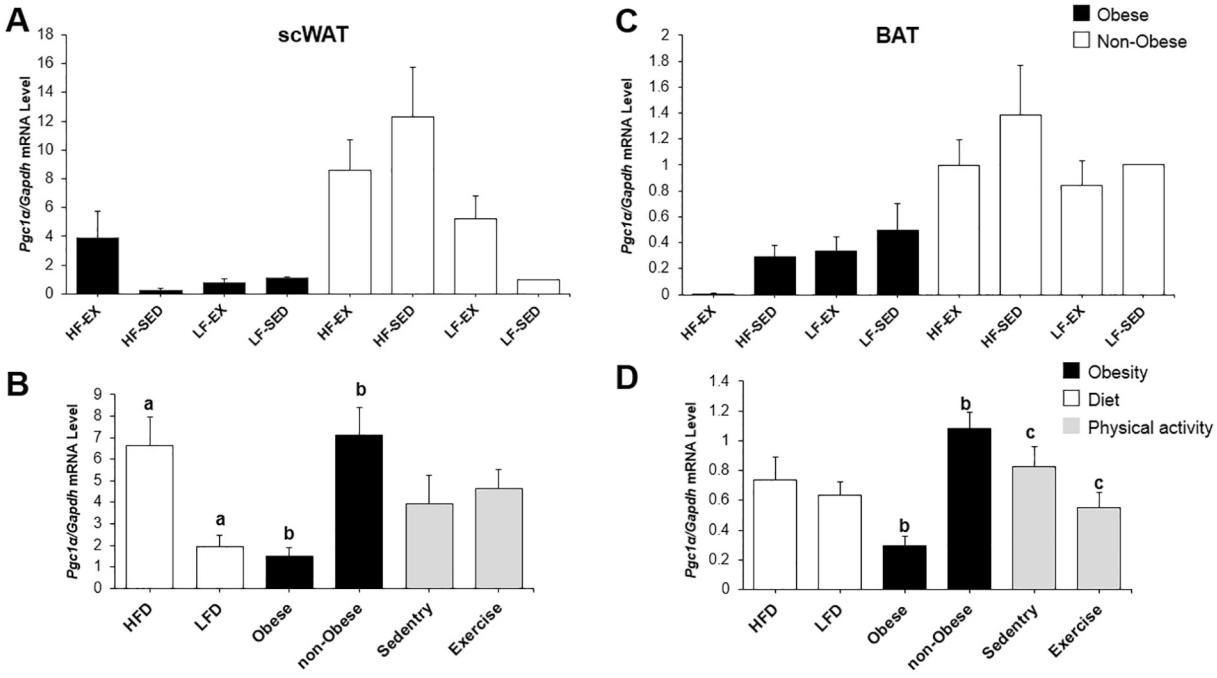

Figure 3. *Pgc1α* mRNA levels in scWAT & BAT.
Pgc1α mRNA expression in scWAT (A) and BAT (C) of mice. Transcript levels of *Pgc1α* were assessed in each group. Obese and non-Obese mice were those mice which treated by HFD and LFD during the first intervention respectively. As elucidated both groups were then divided in 4 groups (in each group, n = 6) during the second intervention as: (HFD/sedentary, HFD/exercise, LFD/sedentary, LFD/exercise). Again, *Pgc1α* mRNA expression in scWAT (B) and BAT (D) of mice based on diet (HFD, LFD), obesity status (obese and no-obese), and physical activity (Sedentary and exercise) are indicated. As obvious, HFD and non-obesity status up-regulated *Pgc1α* levels significantly. Same alphabets indicate significant difference between the same samples ($p < 0.05$).

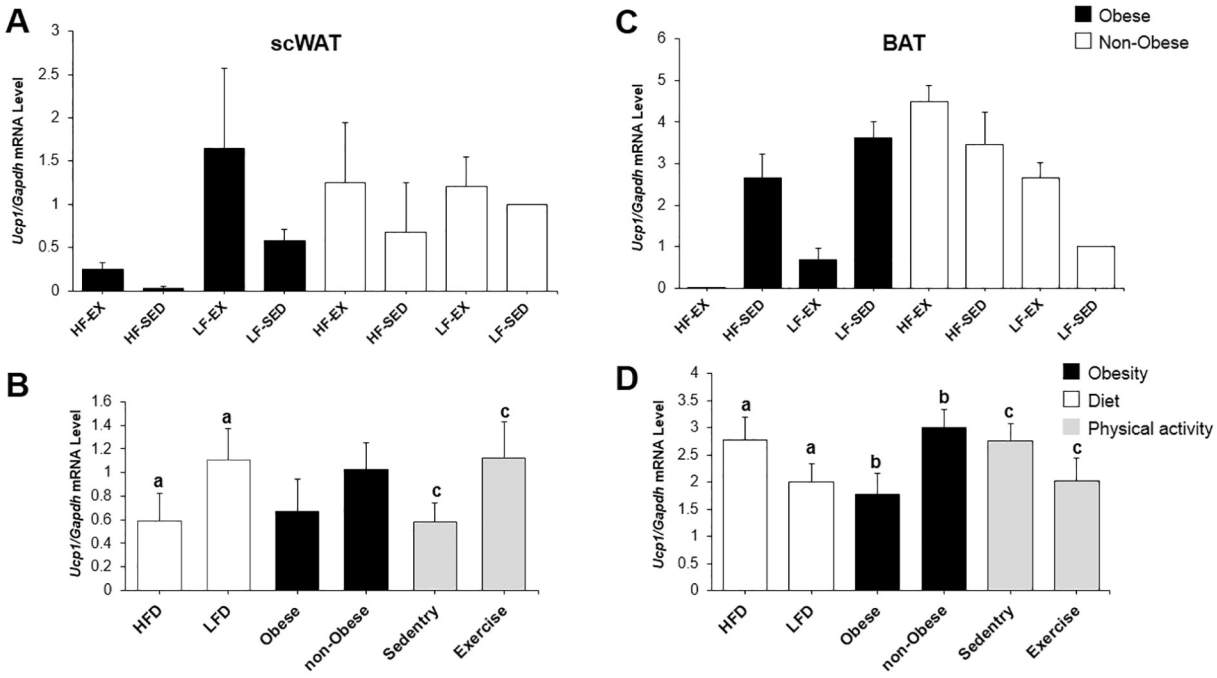

Figure 4. *Ucp1* mRNA levels in scWAT & BAT.
Ucp1 expression in scWAT (A) and BAT (C) of mice. Levels of *Ucp1* mRNA were assessed in obese and non-obese mice were yielded after HFD and LFD during the first intervention respectively. During the second intervention, both groups were then divided in 4 groups (in each group, n = 6); (HFD/sedentary, HFD/exercise, LFD/sedentary, LFD/exercise). Again, *Ucp1* transcripts levels in scWAT (B) and BAT (D) of mice were assessed based on diet (HFD, LFD), obesity status (obese and no-obese), and physical activity (Sedentary and exercise). Importantly, HFD and physical activity excreted controversial modulation in *Ucp1* of scWAT vs. BAT. Same alphabets indicate significant difference between the same samples ($p < 0.05$).

et al., 2014; Koch et al., 2014; Crujeiras et al., 2015; Alvarez-Crespo et al., 2016). As a result in *Ucp1* reduction, BAT would not be able to cause thermogenesis. Obesity even leads to an increase in mitochondrial contents in BAT and scWAT. There are two explanations; first, a rise in the number of adipose tissue cells triggers an increase in number of mitochondria. Second, comparing the weight of adipose

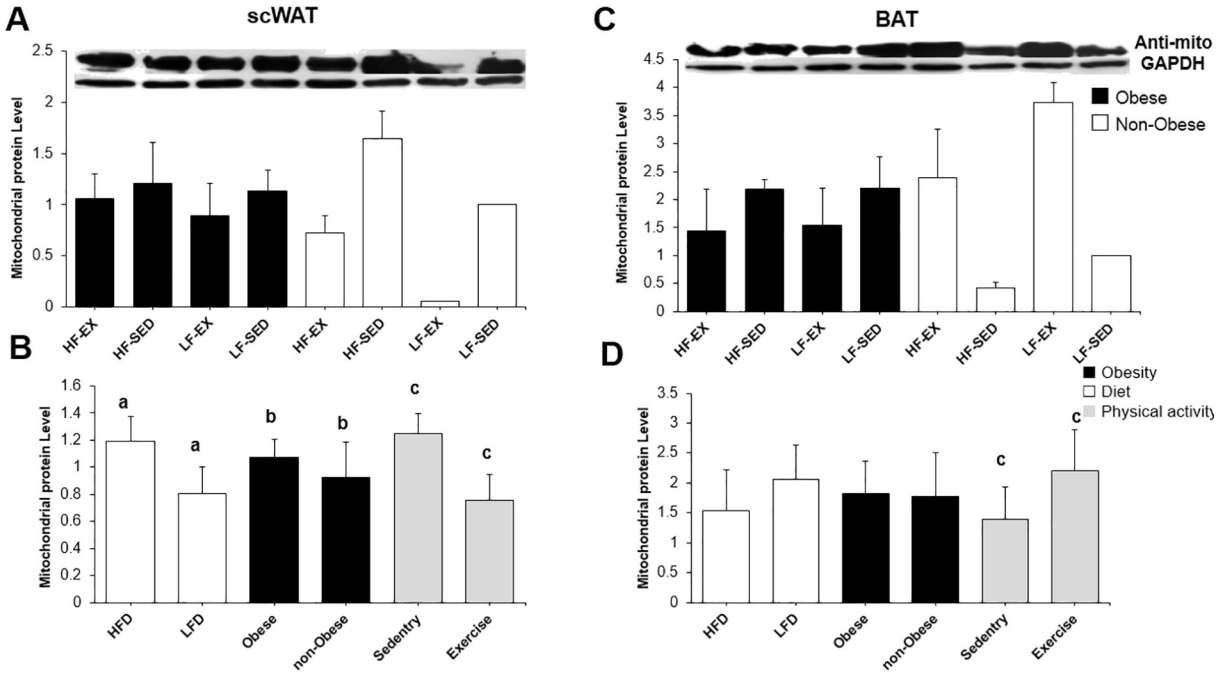

Figure 5. Mitochondrial protein content of scWAT and BAT.
Mitochondrial protein of scWAT (A, B) and BAT (C and D) in 8 groups were assessed by western blotting. As expressed in materials and methods, mice were divided in 8 groups and immunoblotting were carried out on tissue lysates. Upper bands indicate immunoblots with against anti-mitochondria and anti-GAPDH antibodies. Average relative amounts of mitochondrial protein to GAPDH for each group (n = 6) were measured based on the intensity of each band which was quantified by Image J software. To indicate the importance of diet (HFD, LFD), obesity status (obese and no-obese), and physical activity (Sedentary and exercise), mice groups were re-categorized and relative amounts of mitochondrial protein to GAPDH were assessed. Remarkably, HFD, obesity and sedentary situations increased amounts of mitochondrial protein in scWAT similar to physical activity effect on same parameter in BAT. Same alphabets indicate significant difference between the same samples ($p < 0.05$).

tissues and their gene expression indicates that the metabolic efficacy of this tissue has significantly decreased in obesity. In fact, the cell may have a higher mitochondrial amount in order to cope with the decrease in metabolism, but in the presence of obesity and failure to establish the correct mechanisms, the metabolic productivity remains low (Oh et al., 2007). It conclude that obesity create a situation in which the body would not be able to burn calories in form of fatty acids and sever obesity will continue.

In the present study, there was an increase in *Pgc1α* mRNA in response to the HFD in BAT. These results, which are consistent with previous studies, indicate that FFA can exert an effective stimulation on the increase of *Pgc1α* and *Ucp1* (Bargut et al., 2016; Betz et al., 2012; Rachid et al., 2015; Wu et al., 2014) Also, this increase can be due to stimulation of *Fndc5* based on HFD and the endocrine secretion of irisin as a consequence (Morton et al., 2016). However, other studies reported that *Pgc1α* decrease in response to HFD could be due to the type of fatty acids used in the diet, since stimulus of mitochondrial markers just occurs with unsaturated FFAs (Moreno-Santos et al., 2016; Crunkhorn et al., 2007). Unlike the rise in *Pgc1α* the *Ucp1* mRNA reduced in scWAT in response to HFD. FFAs are the main stimulants of *Ucp1* and with a main role in *Ucp1* function cause an elevation in non-shivering thermogenesis (Nedergaard et al., 2001). Nevertheless, decrease in scWAT *Ucp1* might be due to the genetic tendency of the WAT to store FFAs rather than entrance in metabolic pathways. Accordingly, significant effect of HFD on the WAT mass could be due to high amounts of FFAs in a HFD instead of generating non-shivering thermogenesis in scWAT (Bargut et al., 2016). It might indicate that appearance of beige adipose tissue in response to HFD needs more researches because of that we observed *Pgc1α* and mitochondrial content elevation but any *Ucp1* rise, so it could be a great start to use HFD as stimulation in creating beige adipose tissue.

Exercise in mice has led to an increase in scWAT *Pgc1α* and *Ucp1* in consistent with other studies which demonstrate the beige adipose tissue in response to exercise (Morton et al., 2016; Ohyama et al., 2015; Shen et al., 2016; Xu et al., 2011; Stanford et al., 2015; Fain et al., 2013; Trevellin et al., 2014). Also the results have shown a significant reduction in BAT *Pgc1α* and *Ucp1*. Previous studies have demonstrated controversial effects of exercises on BAT activity (Stanford and Goodyear, 2016). However, exercise training down regulates thermogenesis in BAT. Although, it never means that exercise apply no outcome or negative effect on BAT (Betz et al., 2012). Physical activity, as one of the main sources of β-adrenergic stimulation, is one of the important elements in the formation of metabolic pathways in body, serves to improve metabolic disorders (de Queiroz et al., 2012; Kahara et al., 2002). Exercise training has led to a significant increase in scWAT *Ucp1* and increases non-shivering thermogenesis in this tissue as reported earlier (Ringholm et al., 2013; Wu et al., 2014; Shen et al., 2016; Xu et al., 2011). This phenomenon triggers a reduction in WAT mass. Possibly due the heat exertion of training during exercise, the temperature of the trunk increases, therefore BAT decrease the *Ucp1* content to prevent heat injuries (Betz et al., 2012; Brenmoehl et al., 2017). In this study, up regulation in scWAT *Pgc1α*, followed by exercise was associated with an increase in *Ucp1* which determines appearance of beige adipocytes in scWAT of mice due to exercise presumably reflecting endurance training as probable therapeutic approach. In BAT, reduction in *Pgc1α* due to the training was accompanied by a reduction in *Ucp1* mRNA, emphasizing regulatory effect of Pgc1α on *Ucp1* mRNA.

Western blot analysis of mitochondria in BAT and scWAT, stated an increase in mitochondrial protein of BAT in response to exercise training, whereas expression of *Ucp1* and *Pgc1α* down-regulated. In fact, exercises training increased mitochondrial numbers in BAT (Hood, 2009), but the physiological conditions of the body during exercise have not allowed BAT to induce non-shivering thermogenesis. This up-regulation in mitochondrial protein content might exert a positive effect

on FFAs oxidation in sedentary condition which may show another beneficial effect of exercise in this field.

In conclusion, beneficial effect of exercise on increase of mitochondrial content, apearance of beige cells in scWAT would determine that *Ucp1* activation pathways under exercise might be related to its potential therapeutic effects. However, it seems that besides recognized mechanisms, additional pathways might be involved in regulation of *Ucp1* expression.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2018.07.015.

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Author contributions

S. S.: Experimental design, collection and/or assembly of data, data analysis, interpretation and manuscript writing.

F. K.: Experimental design, collection and/or assembly of data, data analysis, interpretation and manuscript writing.

S. M. M.: Conception, design, data analysis, interpretation, manuscript writing and final approval of manuscript.

K. G.: Conception, design, data analysis, interpretation, manuscript writing and final approval of manuscript.

F. E.: Conception, design, data analysis, interpretation and final approval of manuscript.

M. E. and H. S.-E.: Technical assistance and data analysis.

M. H. N.-E.: Conception, design, data analysis, interpretation, manuscript writing and final approval of manuscript.

Conflict of interest

None of the authors has any conflicts of interest to disclose and all authors support submission to this journal.

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