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The effect of 10 weeks interval training on lipocalin 2 gene expression in subcutaneous adipose tissue and lipid profile indices in obese Wistar rats

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ABSTRACT

Background: The purpose of this study is to determine the effect of interval training on lipocalin-2 gene expression in subcutaneous adipose tissue, as well as the serum levels of lipid profile indices (TG, TC, LDL, HDL) in obesed Wistar rats.

Methods: The statistical population consists of all male Wistar rats of the Pasteur Institute of Iran, from which 14 male rats (10-week-old) weighing (220±10 grams) were obesed by 8 weeks of high-fat diet and were randomly divided into interval (n=7) and control (n=7) groups. The exercise group participated in an interval training for 10 weeks, 5 sessions per week in the form of running on a treadmill. The control group did not participate in any exercise program. All rats were dissected 48 hours after the last training session. Finally, after measuring the variables and comparing them by independent t-test between groups.

Results: Interval training resulted in significant decrease in lipocalin-2 expression ($p = 0.029$) and serum TC ($p = 0.022$). Serum HDL was also increased in response to interval training compared to control group ($p = 0.024$). TG ($p = 0.398$) and LDL ($p = 0.658$) remained no change by interval training.

Conclusion: The improvement of the serum HDL and TC in response to interval training in obese rats is probably due to the decrease in lipocalin-2 gene expression in the subcutaneous adipose tissue. Knowing the main mechanisms responsible for these changes requires more studies in this field.

Keywords: Obesity, Interval training, Lipocalin-2 gene expression, Lipid profile, Subcateneous adipose tissue

Introduction

White adipose tissue plays a significant role in causing inflammation and reducing the body's defenses, especially in obesity (1). This tissue, by increasing the amount of subcutaneous or visceral fat, contributes to the inflammatory process in healthy overweight individuals or those with diseases (2,3). Inflammatory factors released from these tissues are important in these processes. Among them, lipocalin 2 is known as one of the important factors of the adipocytokine family and plays a role in various metabolic processes (4). Lipocalin-2 as a peptide derived from adipose tissue, is not only considered a biological marker for various diseases, but also plays an effective role in inflammatory reactions caused by metabolic disorders, and its level is closely related to adipose tissue volume. Also, there is a significant relationship between lipocalin-2 and fatty pancreas, while this relationship is less with fatty liver (4). Lipocalin-2 is involved in the exacerbation of inflammation, obesity, and insulin resistance, metabolic and cardiovascular diseases. Elevated lipocalin-2 levels in obesity and metabolic diseases are associated with the occurrence of coronary artery disease, the severity of atherosclerosis and an increased risk of mortality in patients with heart failure, which points to the importance of lipocalin-2 as a diagnostic marker for cardiovascular diseases associated with metabolic disorders (4).

Reduced lipocalin-2 levels can lead to dyslipidemia, fatty liver disease, and insulin resistance (5). There is a direct relationship between lipocalin-2 levels in the blood and levels of body fat, triglycerides, blood sugar, and insulin resistance (6,7), and an inverse relationship has been observed with HDL levels (7). These findings emphasize the potential importance of

lipocalin-2 in determining lipid profiles and risk factors for cardiovascular disease.

Apart from the direct effect of lipocalins on lipid profile indices, clinical studies have shown that increased lipocalin-2 levels may lead to impaired lipid profile indices in humans (8). According to this evidence, it seems that lipocalin-2 directly or through downstream pathways affects lipid profile indices and consequently cardiovascular risk factors. Therefore, it seems that reducing protein levels or expression of lipocalin-2 through therapeutic interventions may lead to improvement of lipid profile indices. In this regard, the importance of exercise activities is important, although sometimes we see conflicting results. In this regard, it has been reported that 8 weeks of aerobic training in water reduced lipocalin-2, triglycerides, total cholesterol and LDL in obese individuals (9). However, in the study of Ghorban et al (2017), although 8 weeks of resistance training resulted in a decrease in triglyceride, total cholesterol, and LDL levels in obese or overweight men, no significant change was observed in lipocalin-2 levels (10). Despite the aforementioned evidence, which also points to a contradiction in the findings, the role of changes in protein levels or expression of this inflammatory mediator in different tissues in response to different training methods is also less visible on lipid profile indices in different tissues. Therefore, the present study was conducted to determine the effect of 10 weeks interval training on lipocalin-2 expression in subcutaneous adipose tissue and lipid profile indices (TG, TC, LDL, HDL) in obese rats.

Methodology

The statistical population of the present study consists of male Wistar rats from the Pasteur Institute Animal House in Tehran. The study sample consisted of 14 male Wistar rats (10-week-old) weighing (220±10 grams), which were selected from the statistical population and then divided into 2 obesed control (n=7) and interval (n=7) group.

Obesity induction: To induce obesity, a high-fat diet was used for 8 weeks. To prepare high-fat food, 1% cholesterol powder and 1% corn oil (100% pure) were added to the standard rat food purchased from Parsdam Food Company (11).

The rats studied were kept under controlled light conditions (12 hours of light and 12 hours of darkness, light on at 6 pm and light off at 6 am) with a temperature of (22 ± 3 °C) and a humidity range of 30 to 60%. Three rats were kept in Plexiglas cages with mesh doors and dimensions of 25 x 27 x 43 cm so that they had free access to water and fatty food.

Training protocol: Next, rats in the interval training group participated in a 10-week interval training program, 5 sessions per week, in the form of treadmill running with active rest between repetitions (each session consisted of 10 1-minute repetitions with 2 minutes of active rest between them) (12). During this period, the control group did not participate in training (table 1).

Blood sampling and tissue extraction: 48 hours after the last training session (10 to 12 hours of overnight fasting) between 8: am – 9: am, the rats in each group were anesthetized by intraperitoneal injection of a mixture of ketamine (75 mg/kg) and xylazine (10 mg/kg). Then, the animal's chest was opened and a blood sample was taken directly from the heart. Subsequently, a portion of the subcutaneous adipose tissue of the rats was sampled and, after washing in physiological serum, immersed in 1/8 microtubes containing 20% RNAlater liquid and transferred to the laboratory for genetic experiments.

Table 1: Interval training protocol based on speed and time

Weeks	Exercise		Active rest	
	speed (m/min)	Time (S)	speed (m/min)	Time (S)
1 – 2	16	60	10	120
3 – 4	20	60	10	120
5 – 6	25	60	12	120
7 – 8	30	60	12	120
9 – 10	36	60	14	120

** Running time in the exercise phase is 60 seconds and in the active rest phase is 2 minutes and the speed is in meters per minute.*

The concentration of lipid profile indicators (TG, TC, LDL, HDL) was measured by calorimetric method using glucose kit from Pars Azmoun Company-Tehran. Also, RNA was extracted from subcutaneous adipose tissue using RNeasy protect mini kit (QIAGEN) according to the company's instructions. 20 mg of tissue was minced using a scalpel and placed in a microtube, and then RNA was extracted using RNeasy Protect kit according to the instructions of the German manufacturer (13). Table 2 shows the primers pattern.

Statistical analysis: Descriptive statistics were used to describe the data and draw graphs, and independent t-test was used to compare groups in the variables under study. All statistical comparisons were performed using SPSS/Win version 22 software.

Results

Tables 3 summarize the significant within group and between group differences of body weight. No significant difference was observed in body weight at pre-training between groups (p = 0.809). However, significant difference was observed at post-training after interval training between groups (p= 0.001). So, interval training resulted in significant decrease in body weight compared to control group.

Table 4 contains the changes in lipid profiles during the study of 2 groups. Based on output of independent t test, There were no statistically significant differences between groups with regard to TG (p = 0.398) and LDL-cholesterol (p = 0.658). However, significant differences were observed between groups with regard to TC and HDL- cholesterol. So, Interval training resulted significant decrease in TC (p = 0.022) and significant increase in HDL- cholesterol (p = 0.024) compared to control rats.

The statistical result was also showed significant difference in lipocalin-2 expression between groups. So, compared to control group, interval training promoted reduction in lipocalin-2 expression in subcutaneous adipose tissue (p = 0.029, Fig 1).

Discussion

The reduction in lipocalin-2 expression in response to 10 weeks of interval training is one of the main findings of the present study. In other words, 10 weeks of interval training, 5 sessions per week, resulted in a reduction in lipocalin-2 expression in the subcutaneous adipose tissue of obese male Wistar rats compared to the group that did not participate in the training program. These changes were also accompanied by a decrease in serum cholesterol levels and an increase in HDL in response to interval training compared to the control group. Despite these changes, triglyceride and LDL levels did not change significantly.

Table 2: primer sequence

Genes	Primer sequence	Product size	T m	Gene Bank
Lipocalin 2	For: AGCGAATGCGGTCCAGAAAG Rev: GACGAGGATGGAAGTGACGTTG	159 bp	60	NM_001191052.1
RNA PolymraseII	For: ACTTTGATGACGTGGAGGAGGAC Rev: GTTGGCCTGCGGTCGTTC	159 bp	60	NM_001191052.1

Table 3: Pre and post-training of body weight in studied groups (Mean ± SD).

Group	Pre-training	Post-training	p-value (paired t test)
Control	428 ± 26	436 ± 29	*0.001*
Interval	433 ± 31	401 ± 36	*0.001*
p-value (independent t test)	*0.809*	*0.001*	-----

Table 4: Pre and post-training of lipid profile markers of 2 groups (Mean ± SD).

Variable	Control group	Interval group	p-value
Total Cholesterol (mg/dl)	134 ± 11	109 ± 22	*0.022*
Triglyceride (mg/dl)	117 ± 14	112 ± 7	*0.398*
LDL- cholesterol (mg/dl)	130 ± 18	126 ± 17	*0.658*
HDL- cholesterol (mg/dl)	39.7 ± 2.43	43 ± 2.31	*0.024*

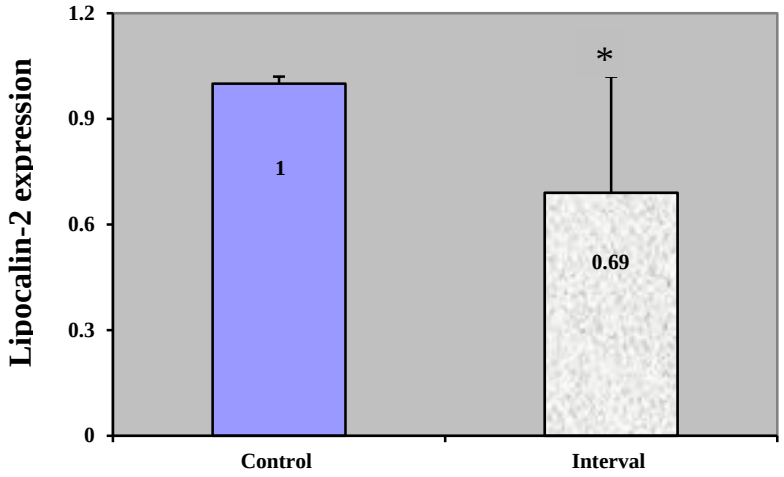

Fig 1: Lipocalin-2 expression in **subcutaneous adipose tissue** of studied groups.

The findings of the present study suggest the effectiveness of interval training on the lipid profile. Although in the present study, LDL and triglyceride levels did not change significantly in response to interval training, this training method led to a significant decrease in cholesterol and a significant increase in HDL compared to the control group. In this regard, some consistent and inconsistent studies are mentioned. As Singel et al (2007), reported no change in LDL following long-term exercise training in morbidly obese individuals (14). However, in the study by Borujeni et al (2014), although 8 weeks of aerobic training was associated with a significant increase in HDL and a decrease in total cholesterol in obese or overweight men with type 2 diabetes, triglyceride and LDL levels did not change significantly (15). On the other hand, studies by Marvicco et al (16), Balduccio et al (2004) and Dunstan et al (2002) have indicated an increase in HDL and a decrease in cholesterol following exercise training (17,18).

In general, most studies have pointed to the positive effect of exercise on profile indicators, which is rooted in several factors such as balance in oxidative stress, changes in hormones, metabolic factors and genetics. In this regard, one of the key factors that helps improve the lipid profile is the reduction of serum levels of inflammatory factors such as lipocalin 2 (6). It has also been said that the reduction of lipocalin 2 in serum and tissue levels, as well as its reduction in expression in target tissues, is associated with an improvement in the lipid profile (7). In this context, in accordance with the results obtained in the present study, Talebi et al (2012) also pointed to a significant reduction in the expression level of the lipocalin-2 gene in the adipose tissue of diabetic mice (19). Also, according to the research of Atashek et al (2017), 8 weeks of resistance training led to a significant reduction in lipocalin-2 in overweight men (20).

However, in the study of Moghaddisi et al (2014), despite the reduction in lipocalin-2 levels following 8 weeks of aerobic and resistance training in healthy, inactive young men, no significant change was observed in CRP levels as a cardiovascular risk factor (8). The reduction in lipocalin-2 levels in response to internal or external factors may depend on changes in other cytokines. In this regard, Samra et al (2009) showed that IL-1B has a regulatory role in the activity and expression of lipocalin-2 in adipose tissue (13). Also, Mehrabani et al (2014) concluded that the reduction in lipocalin-2 in response to aerobic activities is associated with a decrease in IL-1B (21). These studies have shown that the reduction in lipocalin-2 in response to high-intensity exercise (65-75% VO2max) is much greater than that of low-intensity exercise (45-55% VO2max). In addition, the insulin resistance index decreased significantly in both groups under the influence of the exercise program.

To effectively reduce lipocalin-2 in obese individuals, it is necessary to use training intensities exceeding 55% of VO2max (21). Inflammatory cytokines such as TNF-α and INF-γ stimulate the production and release of lipocalin-2 in adipose tissue (22). It has also been reported that lipocalin-2 helps reduce the activity of nuclear factor kappa (NF-kB) by regulating processes related to PPARy receptors and has anti-inflammatory properties (23). The activity

and expression of lipocalin-2 in adipose tissue is influenced by various factors such as insulin, fatty acids, insulin-dependent glucose absorption rate, and cytokines, especially IL-1B, TNF-α, and NF-kβ(24). Although measuring lipocalin-2 gene expression in subcutaneous adipose tissue in response to interval training is considered a strength of this study, the lack of investigation of other variables involved in insulin function is the limitations of the study, and it seems that changes in these variables in response to exercise activities affect the secretion and expression of lipocalin-2 in adipose tissue.

Conclusion

Despite the lack of changes in triglycerides and LDL, but relying on the reduction in cholesterol and increase in HDL, it can be noted that interval training leads to an improvement in the lipid profile or cardiovascular risk factors in obese rats. Considering the effective role of lipocalin-2 in the lipid profile, the improvement of these clinical indicators in response to interval training may be attributed to the reduction in lipocalin-2 expression. Despite the aforementioned evidence, understanding and recognizing the main mechanisms responsible for the effect of exercise training on transcription factors affecting the lipid profile requires further studies.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

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