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Research Article

Effect of Endurance Training and High-Fat Diet on Serum Interleukin-6 and 17 Levels in Male Wistar Rats

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Abstract

Background: High-fat diet (HFD) can cause several health problems and chronic inflammation. The aim of the study was to examine the effect of endurance training along with HFD on serum levels of interleukin-6 (IL-6) and IL-17 in male Wistar rats.

Methods: In an experimental-controlled study, 36 male Wistar rats were randomly assigned to four groups of HFD, training (T), HFD + training (HFDT), and control (C). Exercise training consisted of 40 minutes of running on treadmill at the maximum speed of 55%-65%, five sessions per week for four weeks. Forty-eight hours following the last intervention, serum IL-6 and IL-17 levels were measured. Data analysis was conducted by one-way analysis of variance (ANOVA) and Tukey's post hoc test at $P<0.05$.

Results: The results showed that serum IL-6 and IL-17 levels significantly increased as a result of HFD ($P=0.001$). Endurance training decreased IL-6 and IL-17 levels and there was a significant difference between training and HFD groups ($P=0.001$). However, based on the difference between HFDT and control groups, the variables did not return to baseline values.

Conclusion: According to the results, HFD induced inflammation in a short duration of four weeks manifested by elevated inflammatory indices. In contrast, endurance training ameliorated HFD-induced elevation of IL-6 and IL-17 and these levels did not return to baseline values.

Keywords: Aerobic training, Cytokine, High-fat diet, Inflammation

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Background

Inactivity is a serious health issue in today's life and a principal risk factor for several diseases including obesity, cardiovascular disease, metabolic syndrome, and diabetes (1, 2). Chronic inflammation is a critical determinant in the etiology of many chronic disorders, such as cardiovascular diseases, where circulatory levels of Informatory cytokines increase (3). The associations between the markers including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) with many diseases have been reported (4, 5). For example, CRP and IL-6 levels have been reported to indicate inflammation and be associated with atherosclerosis and other cardiovascular diseases (4). It has also been reported that IL-17 level is related with liver disorders and the development of atherosclerosis (6, 7). Evidence suggests that physical inactivity can independently lead to inflammation and increased blood levels of inflammatory markers (8). Besides, dietary habit is a major factor in the development of diseases (9). Today, the tendency to processed, prepackaged, and high-fat foods is increasing, that is the main cause of metabolic and cardiovascular diseases (10). High-fat diet (HFD) has been reported to result in chronic inflammation and metabolic disorders, including insulin resistance and obesity (11). The HFD-induced inflammation can be reflected by blood levels of cytokines such as IL, TNF-a, and CRP (12, 13).

Evidence shows that regular exercise training, especially aerobic type, protects against metabolic diseases such as obesity and related complications (14). In this regard, aerobic exercise training is the most common type of exercise recommended. According to recommendations by the American College of Sport Medicine (ACSM), weekly exercise of 150 minutes with moderate intensity is required to observe health benefits (14). A negative relationship has been reported between levels of inflammatory markers and fitness levels. Fischer et al reported that endurance training for 10 months decreased IL-6 levels (15). Hewitt et al also reported that moderate-intensity aerobic training decreased concentration of CRP (16). These alterations may be associated with muscle contraction, as evidence suggests that muscle contractions during exercise can regulate the expression and release of inflammatory markers, such as IL-6 and IL-17 (17). Exercise training can cause a substantial change in visceral and subcutaneous fat accumulation and adipose tissue, which are the main active sites for

the production and release of cytokines (18). In addition, IL-6 is known to be released by skeletal muscle during contraction (18).

HFD and regular exercise seem to have apposite effects on the process of inflammation and circulating levels of indicators. Obese individuals show many symptoms of chronic inflammation, such as altered profile of cytokines that are essential for low-grade chronic inflammation (18). For instance, obesity has been illustrated to be negatively associated with IL-15 concentration, while it has a positive correlation with IL-17. Yang et al suggested that HFD elevated IL-15 levels and treadmill exercise training over eight weeks improved obesity and prevented downregulation of IL-15 induced by HFD (19). Thus, regular exercise training may have the potential to improve obesity and HFD-induced changes in cytokine profile. However, it is not clear if exercise training along with HFD can reduce elevated inflammatory markers.

Objectives

This study aimed to examine the effect of endurance training along with HFD intake on the levels of IL-17 and IL-6 in obese Wistar rats.

Materials and Methods

An experimental-controlled design was used in this study. A total of 36 male Wistar rats (age: 10-12 weeks) were housed in a quiet and controlled condition (temperature: 20 ± 2°C, humidity: 50 ± 10%) under a 12-hour light/dark cycle over a 2-week acclimatization. The rats were purchased from Pastor Institute, Iran. Animals then were familiarized with exercise treadmill exercise over a week with incline: 0%, speed: 10–15 m/min, and duration: 5–10 min/d. Following this, an incremental exhaustive exercise test on a motorized treadmill was applied to determine maximal speed. It was a graded exercise test that started with 10 m/min and every 2 minutes the speed increased by 3 m/min until failure. The animals were defined to reach failure if they were not able to keep running in spite of electric shocks. After incremental exercise test, the animals were matched based on their weights and were randomly divided into four groups (n = 9 in each), including HFD, HFD + training (HFDT), exercise training (T), and control (C). The main exercise training program consisted of 40 minutes of running on treadmill at the speed of 55%-65%. Each exercise session commenced with two minutes of warm up with at the speed of 16 cm/s and 0% incline and ended with a brief cool-down. Exercise training program lasted for four weeks and the frequency was five sessions per week.

The HFD groups received daily diet, including 58% fat, 25% protein, and 17% carbohydrate ad libitum (20). The water and food intake of the animals were measured throughout the experimental period. Groups of standard diet were also fed ad libitum with standard rat chow and water (4% fat, 21% protein, 52% carbohydrate, and 13% fiber).

Measurements

Following the interventional period, the animals were anesthetized by ketamine (90 mg/kg) and xylazine (10 mg/kg). Blood samples were collected from left ventricle 48 hours after the last intervention. Then blood samples were centrifuged at 3000 rpm to separate serum samples for assessment of IL-6 and IL-17. Serum samples were then frozen at -40 ° C for later analysis of the variables. IL-6 concentration was assessed by commercial kits (Invitrogen, US) with ELISA assay. IL-17 level was also determined by commercial kits (Cusabio, US) with ELISA assay according to the manufacturer's instruction.

Statistics

Data was present as mean ± SD. Data were first analyzed for distribution by Shapiro-Wilk statistical test. Since the data were normally distributed, one-way analysis of variance (ANOVA) statistical test was used to determine the inter-group differences. When ANOVA reached significance levels, Tukey's post hoc analysis was applied to determine the place of difference. $P < 0.05$ was considered as a statistical difference. Data analysis was performed by SPSS software version 25.

Results

The descriptive data for variables is shown in Table 1. Comparison of baseline values of weight indicated no significant difference across groups at this time point ($P > 0.05$).

According to the results of one-way ANOVA, IL-6 reached a significant level ($P = 0.0001$) indicating a difference across groups. Tukey's post-hoc analysis indicated a significant difference between HFD and control (77% higher vs. control, $P = 0.001$), HFD and HFDT (27% higher vs. HFDT, $P = 0.001$), and HFDT and control (34% higher vs. control $P = 0.001$) (Figure 1). Regarding IL-17, one-way ANOVA revealed an inter-group difference ($P = 0.0001$). Follow-up comparisons by Tukey's statistical

Table 1. Mean ± SD Values for Weight, IL-6, and IL-17 in Groups

Variable	Groups			
	Control	High-Fat Diet	High-Fat Diet + Training	Training
Weight (kg)	301.3 ± 11.2	327.3 ± 10.1	322.2 ± 7.5	294.6 ± 9.7
IL-6 (pg/mL)	36.4 ± 5.1	64.2 ± 12.4	48.2 ± 8.5	31.7 ± 6.2
IL-17 (pg/mL)	51.3 ± 8.1	86.1 ± 16.4	70.2 ± 11.4	47.6 ± 9.3

test indicated a significant difference between HFD and control (68% higher vs. control, $P=0.001$), HFD and HFDT (23% higher vs. HFDT, $P=0.001$), and HFDT and control (37% higher vs. control $P=0.001$) (Figure 2).

Discussion

In this study, we examined the effect of endurance training along with HFD on the levels of IL-6 and IL-17 in male Wistar rats. According to the results, HFD markedly elevated circulatory concentrations of IL-6 and IL-17, but endurance training potentially reduced elevated IL-6 and IL-17. However, the levels of inflammatory variables did not reach the baseline values after exercise training.

According to previous studies, there is a close relationship between Il-6 and IL-17, so that the changes in one complies with the other (7). IL-17 stimulates inflammatory signaling by NF-κB and increases the production and release of IL-6 by the liver and adipose tissue. It also accelerates the progression of inflammation in many diseases, such as alcoholic fatty liver. In addition, the expression of IL-6 in the liver is associated with the severity of inflammation, as well as fibrosis in conditions such as non-alcoholic fatty liver disease (7). Evidence shows that HFD can enhance the expression and release of inflammatory markers such as IL-6 (21). Diets with high fat content increase the fat accumulation in visceral and subcutaneous adipose tissue and at bone marrow, which in turn increases the release of inflammatory cytokines (21).

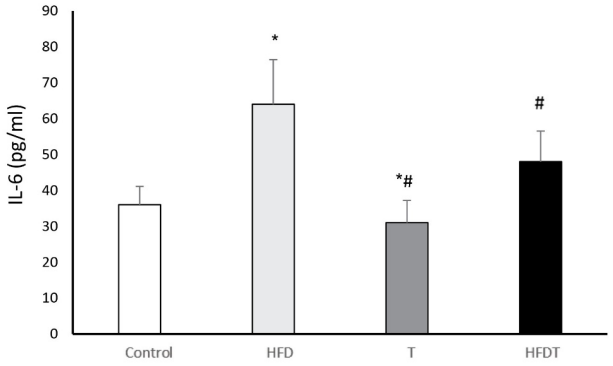

Figure 1. Mean ± SD for IL-6 in groups. * Indicating a significant difference ($P=0.001$) with control # Indicating a significant difference ($P=0.001$) with HFD.

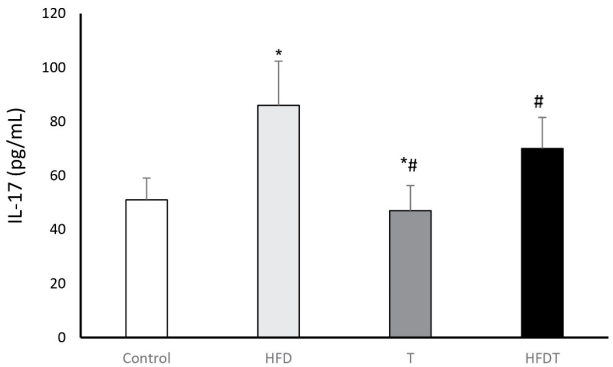

Figure 2. Mean ± SD for IL-17 in the Groups. * Indicating a significant difference ($P=0.001$) with control. # Indicating a significant difference ($P=0.001$) with HFD

Although this is the first study investigating effect of endurance training along with HFD intake on some inflammatory markers, our results seem to be consistent with some previous studies (22, 23). Wang et al indicated that endurance training had beneficial effects on indicators of metabolic syndrome and improved IL-6 levels in patients with metabolic syndrome (22). Kohut et al also reported that endurance training improved IL-6 and CRP levels in older adults independent of weight changes (23). These findings support our results that endurance training has the potential to alleviate inflammation induced by diets with high fat content. A possible explanation to this effect could be the changes in visceral fat mass following regular exercise training as these are mainly released by adipose tissue (24, 25). This was confirmed by Jae et al, reporting a close relationship between body fat mass and levels of inflammatory markers (26). Thus, elevated energy expenditure through regular endurance training reduces visceral fat and may regulate the release of inflammatory cytokines. Also, evidence suggests that sympathetic activation increases cytokine release from adipose tissue. Endurance training can reduce sympathetic stimulation and thus reduce the cytokine release of adipose tissue.

However, the results of this study were inconsistent with those of Libardi et al, demonstrating that following 16-week aerobic, resistance, and concurrent training in sedentary middle-aged individuals, there was no significant change in the levels of inflammatory indices (27). In addition, Eaton et al. reported that high-intensity interval training increases resting levels of IL-6 (28). This discrepancy might be partly explained by the time period between the last exercise session and collection of blood samples. In the study by Libardi et al, the final blood sample was collected seven days following the last exercise session, and in the study by Eaton et al the final blood samples were taken just three hours after exercise intervention (27, 28). It has been revealed that acute exercise increases the release of inflammatory indices (29). In addition, the difference in participants and baseline values of the variables can also explain the discrepancy, so that in the research by Libardi et al, the participants were healthy individuals with normal baseline values of the inflammatory cytokines (27). Furthermore, other determinant factors for the effect of exercise on inflammatory cytokines include the type, intensity, and duration of exercise intervention. High-intensity exercise elevates markers of inflammations to a greater extent than low- to moderate-intensity exercise. Eaton et al. applied high-intensity exercise that can explain elevated levels of cytokines (28).

This study had some strengths and limitations to be acknowledged. Administration of HFD is against ethics of human research; hence, this study was conducted

on animal models to determine direct effect of exercise training during HFD intake. Also, we did not assess glucose concentration, that might be a limitation as it is associated with metabolic disorders and inflammation during obesity. Moreover, assessment of indicators of acute phase inflammation such as CRP would have provided further insight on the effects of these two interventions in combination.

Conclusion

Overall, our results revealed that HFD intake increased the circulating levels of IL-6 and IL-17 in male Wistar rats. In contrast, endurance training had the potential to reduce the elevated levels of inflammatory markers. However, exercise training was not as effective to reverse HFD-induced elevation of IL-6 and IL-17 to baseline levels.

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Conflict of Interests

The authors have no conflict of interest to declare.

Ethical Approval

The study procedures complied with the codes of Helsinki Declaration for animal research and was approved by the institutional committee of Research Ethics Committee of Islamic Azad University (Ethics No. IR.IAU.TABRIZ.REC.1397.022)

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