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Effect of High Intensity Interval Training with Flaxseed on Interleukin-1 Beta and Lipocalin-2 Gene Expressions in the Heart Tissue of Rats

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Abstract

Background: Inflammation plays an important role in the development of atherosclerosis. Interleukin-1 beta (IL-1β) and lipocalin-2 (LCN-2) are important inflammatory markers in the pathogenesis of atherosclerosis.

Objectives: This study aimed to investigate the interactive effects of high intensity interval exercises (HIIT) with flaxseed oil consumption on IL-1β and LCN-2 gene expressions in the heart tissue of rats.

Methods: In this experimental study, 20 adult male Wistar rats were randomly selected and divided into four groups of five rats: (1) Control, (2) HIIT, (3) 20 mg/kg of flaxseed oil, and (4) HIIT with mg/kg 20 flaxseed oil. Groups 2 and 4 ran on a treadmill for 10 weeks and five weeks each, with 90% to 95% intensity of VO_{2max}. For analysis of the research findings, Kolmogorov-Smirnov statistical analysis and two-way ANOVA were used ($P \leq 0.05$).

Results: Ten weeks of HIIT (P = 0.001) and flaxseed oil consumption (P = 0.03) had a significant effect on reducing the LCN-2 gene expression in the heart tissue of rats. However, 10 weeks of HIIT (P = 0.19) and flaxseed oil consumption (P = 0.19) had no significant effect on IL-1β gene expression in the heart tissue of rats. In addition, 10 weeks of HIIT with flaxseed oil has no interactive effects on IL-1β (P = 0.91) and LCN-2 (P = 0.99) in the heart tissue of rats.

Conclusions: Although it seems that the simultaneous use of flaxseed oil with HIIT does not have interactive effects on IL-1β and LCN-2 gene expressions in the heart tissue of rats, the use of flaxseed oil and HIIT alone results in improved LCN-2 gene expression in the heart tissue of rats.

Keywords: Training, Flaxseed Oil, Heart, IL-1β, LCN-2

1. Background

Inflammation plays an important role in the development and advancement of atherosclerosis. Increased levels of inflammatory factors can lead to leukocyte adhesion to the endothelium of the vessels and ultimately cause atherosclerosis (1). Lipocalin-2 (LCN-2) is a cytokine that belongs to the lipocalin family and acts as an inflammatory cytokine on signaling pathways. The expression and secretion of lipocalin-2 is induced by many inflammatory stimuli, including interleukin-1 beta (IL-1β) (2-4). Research has shown that IL-1β, with its inflammatory properties, reduces cellular function and is associated with an increase in LCN-2 (5, 6). On the other hand, it has been shown that inflammatory mediators such as LCN-2 and IL-1β are associated with the development and advancement of various diseases, including cardiovascular disease, kidney damage, intestinal inflammation, and cancer (7). From ancient times, plants have played an important role in the treatment of many diseases. It has also been noted that Iranians have been pioneering people in the use of medicinal herbs (8). Flaxseeds contain omega-3 and omega-6 fatty acids and research has shown that flaxseed oil has many biological agents, including anti-inflammatory and anti-oxidant effects; in addition, omega-3 and omega-6 fatty acids in flaxseed oil prevent diseases caused by inflammation, including cardiovascular disease (9). Accordingly, studies today attribute this characteristic of omega-3 to its anti-inflammatory effects (10). A study found that flaxseed oil reduced oxidative stress and reduced the incidence of inflammation in dialysis patients (11). In another study, it was found that flaxseed oil reduced the C-reactive protein (CRP) in obese subjects (12). Few studies have been conducted on the effect of flaxseed oil on the concentration of inflammatory factors. One study found that a 10-week flaxseed diet reduced the levels of IL-1β and TNF-α in male Wistar rats (13). Another study also found that a flaxseed oil consumption period reduced the concentration and gene expression of the intercellular adhesion molecule-1 (ICAM-1), an agent of inflammatory mediators (14). Regarding the effect of exercises on immune response, studies have shown that there is an inverse relationship between levels of physical activity with inflammatory mediators, with a high level of physical fitness with low levels of inflammatory mediators (13-15). It has also been shown that high intensity interval training, in addition to reducing the amount of time and level, has a similar effect on health compared to endurance training (16). Accordingly, the results of the study showed that IL-1β levels in rats decreased after a mandatory exercise course on the treadmill (17). In another study, 16 weeks of exercise training significantly reduced the level of IL-1β (18). Another study also found that exercise had no effect on inflammatory mediators such as IL-1β (19, 20). Few studies have been conducted on the effect of physical activity on lipocalin, thus, in a study, the effect of a treadmill training period on retinol binding protein-4 (RBP-4) levels in male rats was evaluated, which resulted in reduced levels of RBP-4 visceral fat tissue after the exercise period, though the LCN-2 was not monitored (RBP4 belongs to the lipocalin family) (21). In another study, the effect of high intensity interval training (HIIT) on the gene expression of inflammatory biomarkers was investigated and the results showed that high HIIT reduced serum levels and gene expression of IL-1β and LCN-2 in fast and slow contraction muscles (22). Still again, in another study, it was found that aerobic training significantly inhibited the increase of IL-1β gene expression from particulate matter 10 (PM10) in male rats (23). Regarding the simultaneous effect of sports exercises and flaxseed oil supplementation on IL-1β and LCN-2 factors, there are very few studies. In one study, it was found that flaxseed oil consumption along with exercise training decreased IL-1β and TNF-α levels in rats with ischemic heart disease (24). In another study, exercise training and flaxseed oil consumption reduced the concentration of IL-1β and TNF-α (13).

2. Objectives

Regarding the contradictions and the role of inflammatory factors in the development of diseases and the limited research on the effect of flaxseed oil on LCN-2 gene expression, as well as the limited studies on the effect of HIIT on LCN-2, this study aimed to investigate the interactive effects of HIIT combined with flaxseed oil consumption on IL-1β and LCN-2 gene expression in the heart tissue of rats.

3. Methods

In this experimental study, 20 Wistar rats with a weight range of 323.7 $\pm$ 29.07 grams and 18 weeks old were selected from the animal house of Baqiyatallah University of Medical Sciences, based on availability, and were assigned as the statistical sample of the study. Rats were kept at 22 $\pm$ 2°C, 45% - 50% humidity and dark-light cycle (12 hours of light and 12 hours of darkness). Before performing the research, the rats were allowed to adapt to the laboratory environment and were then randomly divided into four groups of five rats: (1) Control, (2) HIIT, (3) 20 mg/kg flaxseed oil, and (4) HIIT 20 with mg/kg flaxseed oil. The food of rats was provided from Karaj Behparvar Animal Food Company, which rats were freely availed with it. To prepare flaxseed oil, freshly ground flaxseeds were provided from Mehriz city of Yazd province, and after drying, the oil was extracted using oil extraction apparatus and fed to rats in appropriate doses.

The training groups performed the relevant trainings at the specific time for ten weeks, each week for five sessions, including running with 90% to 95% intensity of VO_{2max} on the rodents' treadmill, and at the same time, the control group was placed on a treadmill for 15 minutes with a speed of 2 m/min to equalize the effect of stress. It should be noted that the HIIT protocol included three stages of warming-up, the main body of the training (three intervals), and cooling. The trainings were performed in a 6 minute warm-up phase with 50% to 60% intensity of VO_{2max}, in the main body phase, 4 minute high intensity interval trainings with 90% to 95% intensity of VO_{2max}, 2 minute low intensity interval training with 50% to 60% intensity of VO_{2max}, and training in the cooling stage was done for 6 minutes with 50% to 60% intensity of VO_{2max}. The training protocol lasted up to 72 hours before sacrificing the rats. At the end of the research period, rats were sacrificed and their heart tissue was removed.

For molecular analysis at the gene expression level, at first, the extraction of RNA was carried out, according to the manufacturer's protocol (CinnaGen, Iran). Then, using the light absorption property at 260 nm, with the aid of the following relationship, the concentration and degree of purity of the RNA sample was quantitatively achieved.

$C\ (\mu g/\mu L) = A260 \times \varepsilon \times d/1000$

C: A260 concentration: Optical absorption at 260 nm wavelength; ε: Molar offset for RNA at 40 and for DNA at

50, d: Dilution factor.

After extracting RNA with high purity and high concentration from all of the samples, cDNA synthesis steps were taken according to the manufacturer's protocol (Fermentas, USA) and then, the synthesized cDNA was used for conducting reverse transcription reaction. Initially, the designed primers for genes were examined and genes were examined by quantitative q-RT PCR method.

- IL-1β

-F: TAC CTA TGT CTT GCC CGT GGA G

-R: ATC ATC CCA CGA GTC ACA GAG G

- LCN-2

-F: GGA ATA TTC ACA GCT ACC CTC

-R: TTG TTA TCC TTG AGG CCC AG

Data on mean and standard deviation were reported. The Shapiro-Wilk test was used to evaluate the normal distribution of the findings, and, as the distribution of the findings was normal, two-way analysis of variance was used to compare the levels of research variables in the four groups. The significance level was considered to be 0.05 in all statistics.

4. Results

The levels of LCN-2 and IL-1β gene expressions in the heart tissue of four groups of study are presented in Table 1. The results of Shapiro-Wilk test showed that distribution of the findings was normal. The results of the two-way ANOVA test in Table 2 shows that 10 weeks of HIIT (F = 17.01; P = 0.001 and effect size = 0.51) and flaxseed oil consumption (F = 5.47; P = 0.03, and effect size = 0.25) have a significant effect on the reduction of LCN-2 gene expression in the heart tissue of rats. However, HIIT with flaxseed oil has no interactive effects on the reduction of LCN-2 gene expression in the heart tissue of rats (F = 0.001; P = 0.99 and effect size = 0.001). In addition, 10 weeks of HIIT (F = 1.85; P = 0.19, and effect size = 0.10) and flaxseed oil consumption (F = 1.85; P = 0.19 and effect size = 0.10) has no effect on the IL-1β gene expression in the heart tissue of rats. Besides, HIIT with flaxseed oil has no interactive effects on the IL-1β gene expression in the heart tissue of rats (F = 0.01; P = 0.91 and effect size = 0.001).

Table 1. LCN-2 and IL-1β Gene Expressions in the Heart Tissue of Four Groups of Research[a]

Group	Variable	
	LCN-2	IL-1β
Control	0.6483 ± 0.03543	0.2308 ± 0.05301
HIIT	0.5715 ± 0.04802	0.2018 ± 0.03873
Flaxseed oil consumption	0.6048 ± 0.04276	0.2018 ± 0.04290
HIIT with flaxseed oil	0.5276 ± 0.03968	0.1773 ± 0.03934

[a]Values are expressed as mean ± SD.

5. Discussion

The beneficial effects of exercise on metabolic parameters have been reported in many studies. However, there is little evidence of its impact on the immune system and inflammatory parameters in the heart tissue. Regarding the effect of exercise activities on immune responses, there is an inverse association between inflammatory biomarker concentrations and physical activity level, meaning that in subjects with high physical activity and high physical fitness, inflammatory factors are significantly lower than those with no activity or low physical fitness (25). For this purpose, the present study was designed to determine the effects of 10 weeks of HIIT with flaxseed consumption on LCN-2 and IL-1β gene expression in myocardial tissue of healthy adult rats. Regarding the values of LCN-2 and IL-1β gene expressions, the results of the study indicated a significant decrease in LCN-2 in the HIIT group, flaxseed consumption, and the interactive group of HIIT and flaxseed consumption. However, the HIIT, the flaxseed consumption, and the HIIT with flaxseed consumption, had no significant effect on IL-1β levels. The potential reason for non-significant impact of combination of HIIT and flaxseed consumption could be due to different issues such as small sample size, low consumption dose of flaxseed consumption, or short research period. Virtually, it could be due to the heterogeneity of the impact of this combination within the group that made dispersion for the variable and reduce the power of test. There are limited results regarding the effects of exercise on LCN-2 and IL-1β. In this regard, Mansouri et al. evaluated the effect of 7 week treadmill training on RBP-4 values in male rats. Their training protocol consisted of 20 minutes of training at a low speed of 15 - 20 m/min for 5 days a week, then, the duration and intensity of the training increased, thus, in the last 2 weeks the rats trained for 35 minutes and 30 m/min. They showed that following these trainings, RBP-4 levels of visceral and muscular fat in rats decreased (21). However, this study was conducted on one of the LCN-2 families; LCN-2 was not directly evaluated and the targeted tissue to be evaluated was not coronary heart disease.

In addition, in regards to human studies (on serum samples), Choi et al. evaluated the effect of 12 weeks of aerobic exercise training on LCN-2 serum in obese women. In this research, exercise activities consisted of 45 minutes per day and resistance training included 20 minutes per day of exercise. The results indicated no change in LCN-2 after 12 weeks of aerobic and resistance training (26). In

Table 2. Results of Two-way Analysis of Variance to Investigate the Effect of HIIT and Flaxseed Oil Consumption on LCN-2 and IL-1β Gene Expressions in the Heart Tissue of Rats

Variable/Factor	Sum of Squares	df	F	P Value	Effect Size
LCN-2					
HIIT	0.03	1	17.01	0.001	0.51
Flaxseed oil	0.01	1	5.47	0.03	0.25
HIIT and flaxseed oil interaction	1.31	1	0.001	0.99	0.001
Total	6.98	20			
IL-1β					
HIIT	0.004	1	1.85	0.19	0.10
Flaxseed oil	0.004	1	1.85	0.19	0.10
HIIT and flaxseed oil interaction	2.50	1	0.01	0.91	0.001
Total	0.86	20			

contrast to the above studies, Bijeh and Abbasian showed that endurance training for 4 weeks and 50% to 75% intensity of VO_{2max} could significantly reduce serum LCN-2 levels (27). However, the discussion of the results of the present study should be done extremely carefully. This is due to the fact that in the study of Mansouri et al. RBP-4 (a member of the LCN-2 family) as well as adipose and muscle tissues were used and other studies were conducted on human subjects (21). In this regard, Lovatel et al. also evaluated the IL-1β values in the hippocampus of male rats after training on the treadmill. Their results indicated that compulsory training on the treadmill was associated with a decrease in IL-1β in the hippocampus of male rats (17). In addition, Agarwal et al. assessed the effect of 16 weeks of exercise training on the levels of IL-1β in male rats. Their results also showed a significant decrease in IL-1β values in male rats' brain (18). Speisman et al. also evaluated the effects of 12-week cycling training on IL-1β male rats. Their results indicated a reduction in IL-1β values in rats' brains (28). Regarding the above items, it is likely that the reduction of LCN-2 gene expression in the present study results from a decrease in the amount and activation of NF-kB due to exercise activity. Although the exact mechanism for reducing LCN-2 gene expression in the present study is not well known, it may indicate the adaptive adjustability and anti-inflammatory nature of sport activity. On the other hand, myocardial inflammation plays a pivotal role in the functioning of the heart and its disorders (29) and is one of the common characteristics in CVDs (30). Inflammation is a potent inducer of oxidative stress, and NF-kB responds to inflammation and induces oxidative stress (31). Oxidative stress and inflammation share common and corporate signaling pathways. ROS can trigger inflammation through damage to macromolecules. ROS is also a product of the inflammation process (32). Several transcription factors play an important role in the development of myocardial inflammation and CVDs (33). Nuclear factor-kappa B (BNF-kb) is a polytropic transcription factor associated with the pathology of many heart diseases. Oxidative stress causes the transfer of NF-kb to the nucleus and the transcription of several pathogenic genes (34) and mediates the expression of a number of genes from the pro-inflammatory cytokines (29, 33). NF-Kbp65 is one of the most important family members of NF-Kb and plays an important role in the development of disease (33). Modulating the inflammatory response is a new and promising way of treating CVDs (35).

Recently, it has been found that vitamin D, omega-3, and omega-6 play an important role in modulating the immune/inflammatory system (36). Omega-3 and omega-6 play an important role in inflammatory system modulation, which is done by regulating the production of inflammatory cytokines and inhibiting the proliferation of pro-inflammatory cells, both of which play a determining role in the pathogenesis of inflammatory diseases (36-38). Omega-3 also inhibits NF-kB activity by suppressing NF-kB nuclear transmission and its transcription activity (37). Inhibition of NF-kB activation is an effective strategy to prevent the production of inflammatory and pro-inflammatory cytokines. The suppression of NF-kB activation is involved in reducing inflammation of the heart (29, 30). The training activity also positively regulates the antioxidant defense and proteins reconstruction mechanisms in the body through redox sensitive transcription factors, including NF-kB. A temporary and transient increase in the ROS levels activates the NF-kB cascade. The NF-kB signal cascade is positively correlated with antioxidant defense mechanisms to counteract and prevent the formation of defective inflammatory cycles and oxidative stress associated with cardiovascular disease (32). A review of the related literature cannot provide a proper and accurate evidence of the effect of different intensities of aerobic exercise on the concentration of LCN-2 and IL-1β. As noted above, there has been little research on the effect of physical activity on these adipocytokines, and extremely little research on LCN-2. In this regard, Choi et al. examined the effects of exercise on LCN-2, which resulted in no significant changes in LCN-2 concentrations after 12 weeks of moderate-intensity exercise in obese women (26). Therefore, this hypothesis is likely to be raised that the main reason for the ineffectiveness of their program has been the intensity of exercise, and it seems that a higher intensity exercise may reduce the levels of this adipocytokine. On the other hand, due to the fact that the intensity of exercise is the main factor affecting the outcome of the exercise, the assessment of the effect of exercise intensity on the blood levels of these variables is important. There is also the hypothesis that the greater the amount of energy consumed during exercise and the higher metabolic pressure on body organism, the more evident the lowering of the fat mass is (39), which may potentially affect LCN-2 levels in the bloodstream. Whether the change in exercise intensity affects inflammatory, adipocytokines has been studied on some of them (39). However, the effect of exercise intensity on LCN-2 and IL-1β blood levels requires further evaluation. LCN-2 has been named as a mediator of obesity and insulin resistance,and other metabolic disorders associated with obesity (2, 4). The same link between obesity and the incidence of inflammation and chronic diseases seems to have raised LCN-2 as an inflammatory marker for researchers. The results of this study showed that the values of this adipocytokine significantly decreased in the training group, however, in the study of de Graaf-Roelfsema et al. which was performed less intensely than the present study, there was no significant change, despite the decrease. Perhaps this decrease is attributed to the nature of the exercises with a higher intensity, which although it causes inflammation in the short term (40), it has anti-inflammatory effects in the long term and decreases the risk of insulin resistance by decreasing the fat mass. Since regular physical exercises have a positive effect on other inflammatory markers and cardiovascular risk factors, such as triglyceride, low density lipoprotein, and total cholesterol (41), the reason why levels of this cytokine have not diminished after moderate intensity exercise is likely to be due to other factors, including hormonal changes and substrate metabolism that have not been addressed in this study. Given that excessive oxidative stress and inflammation (42) contribute to the development of chronic diseases, the use of auxiliary agents in efforts to minimize oxidative stress may be helpful. This is especially true for people who are typically involved in extreme sports activities as an ergogenic factor. Fatty acids alleviate the effects of free radicals in two ways. First, omega-3 fatty acids may increase catalase levels in peroxisomes and cytoplasm, thus, improving defense against free radicals. Second, omega-3 fatty acids are replaced by fatty acids that are attacked by oxygen radicals (43, 44). In addition, omega-3 consumption has been shown to reduce urine F2 isoprostane as an oxidative stress biomarker. In vivo findings on the mechanism of omega-3 activity led to a series of studies that directly demonstrated inhibitory effects of docosahexaenoic acid on NOX, that is, NOX2 and NOX4 (45). Therefore, since cardiovascular disease and oxidative stress are a major problem people face, and physical activity increases the antioxidant defense system by reducing oxidative stress. In addition, considering the positive effects of exercise activity and antioxidant supplements, omega-3 and omega-6 alone, on the formation of defective inflammatory cycles, the use of these antioxidant supplements is likely to enhance the effect of exercise activity.

5.1. Conclusion

According to the results of this study, it can be concluded that although the use of flaxseed oil simultaneously with HIIT does not have interactive effects on IL-1β and LCN-2 in the heart tissue of rats, the use of flaxseed oil and HIIT alone can improve the LCN-2 gene expression in the heart tissue of rats.

Footnotes

Authors' Contribution: All authors equally contributed to the writing and revision of this paper.

Conflict of Interests: There was no conflict of interest.

Ethical Considerations: For ethical considerations, animal experimental procedures were in accordance with institutional guidelines and approved by the Ethical Committee of Laboratory Animals Care at Marvdasht Islamic Azad University, Marvdasht, Iran. (IR.MIAU.REC.1396.163).

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