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The effect of Eight Week Aerobic Training with Omega-3 Fatty Acids Supplementation on Paraoxonase and Myeloperoxidase Levels in Sedentary Women

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ABSTRACT

Introduction: The effectiveness mechanism of aerobic training and omega-3 fatty acids supplement alone or combined together is remarkably unknown. In the present study, the effect of eight weeks aerobic training with omega-3 fatty acids supplementation on the levels of paraoxonase and myeloperoxidase in the sedentary overweight and obese women have been investigated.

Materials and Methods: Forty sedentary young women with age ranging 25-40 years were randomly divided into four equal groups (10 subjects in each group) including placebo (P), omega-3 fatty acids supplement (O), aerobic training (T), and aerobic training + omega-3 fatty acids supplement (TO) groups. The subjects in the T and TO groups participated in aerobic training for eight weeks, three session per week, and the TO and O groups consumed omega-3 fatty acids supplement in daily 2000 mg dose. Blood samples collected before and after eight weeks intervention and the level of paraoxonase, myeloperoxidase and some other related metabolites was determined.

Results: Serum levels of paraoxonase in the O, T and TO groups significantly increased compared to the P group ($P < 0.001$). Myeloperoxidase indicated a significant decrease in T and TO groups compared to P and O groups ($P < 0.05$). Moreover, significant increase of Apo-A1 and significant decrease of Apo-B were observed in both trained (T and TO) groups ($P < 0.05$).

Conclusion: Despite increasing the paraoxonase levels following omega-3 fatty acids supplementation, the supplementation in combination with the aerobic training did not exert synergistic effect in the studied variables compared to the aerobic training alone.

Keywords: Aerobic training, Sedentary women, Paraoxonase, Myeloperoxidase

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Introduction

Overweight and obesity negatively affects almost all body physiological functions, and has been considered as a serious health challenge worldwide (1). Obesity, and especially the accumulation of visceral fat, is associated with insulin resistance, hyperglycemia, dyslipidemia, and hypertension, and increase the risk of developing type 2 diabetes mellitus and cardiovascular disease, thereby increase the rate of mortality (2). According to available evidence, obesity is associated with changes in the levels of some circulating factors. It has been reported that paraoxonase-1 (PON-1) levels have a negative correlation with obesity and its levels in obese people are significantly lower compared to normal weight individuals (3). Paraoxonase-1 is a major protein associated with HDL-c, which is highly lipophilic and, along with apolipoproteins A-1 and Apo-J, is present in HDL particles, which inhibit LDL oxidation and reduce oxidative stress in the arteries (4).

Paraoxonase-1 dysfunction in the obese individuals result in increased the insulin resistance and plays an important role in the development of metabolic syndrome (5). Paraoxonase-1 levels decrease in patients with atherosclerosis, type-2 diabetes, and patients with cardiovascular disorders (6). Paraoxonase-1 is mainly produced by the liver and in addition to its role in the counteract with atherosclerosis and heart disease, it has also been widely considered as an anti-inflammatory mediator (7). In fact, paraoxonase-1 binds to high density lipoprotein (HDL-c) in human subjects and inhibits the oxidation of lipoproteins, decreased the degree of inflammation, therefore, paraoxonase-1 considered as an anti-atherosclerotic factor (8). Some researchers have also identified the Paraoxonase-1/myeloperoxidase (MPO) ratio as a risk factor for cardiovascular disease (9).

Previous studies have shown that myeloperoxidase is a hemoprotein that binds to HDL-c, which is associated with oxidative stress and an increased risk of atherosclerosis, and myeloperoxidase is one of the major enzymes involved in accelerating lipid peroxidation in plasma (10). It has been reported that various factors, including exercise training (11) as well as omega-3 supplementation (12, 13) can affect PNO-1 and MPO levels. Epidemiological studies have shown that omega-3 supplementation prevent the atherosclerotic diseases, and based on the available evidence, it seems that positive effects of omega-3 supplementation are exerted through its role in improving endothelial dysfunction, and it's suggested that healthy people without cardiovascular disease can benefit from the preventive benefits of this supplement against cardiovascular disease (14). It's reported that aerobic training leads to a significant increase in paraoxonase-1 levels in obese men, but showed no significant change in paraxonase-1 levels with resistance training (15). In addition, some researchers have reported that eight weeks aerobic training (three sessions per week) in sedentary men don't have a significant effect on the paraoxonase activity (16). In addition, a significant increase in paraoxonase levels and a decrease in some cardiovascular risk factors such as CRP have been reported following omega-3 supplementation (13).

Despite conducted studies, the findings are contradictory, and simultaneous effect of exercise training and omega-3 on the levels of paraoxonase and myeloperoxidase is still unknown. Therefore, the aim of present study was to investigate the effect of aerobic training with omega-3 ingestion on the levels of paraoxonase and myeloperoxidase levels in overweight and obese women.

Materials and Methods

Subjects

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The subjects the present study consisted of overweight and obese women with age ranging from 25 to 40 years, were chosen to conducting the study protocol. Subjects' age, height, body weight and boddy mass index (BMI) in the placebo, omega-3, aerobic training, and aerobic training + omega-3 supplement groups were reported in Table 1. The study was conducted according to the principals of the Declaration of Helsinki and its protocol was approved by the ethics committee of Islamic Azad University- Science and Research Branch (Ethical codes: IR.IAU.SRB.REC.1399.010).

Table1. The characteristics of different groups of the subjects participated in the study.

Characteristic	TO	T	O	P	TO
Age (years)	27.81 ± 3.35	26.26 ± 2.98	26.74 ± 3.19	28.31 ± 3.63	27.82 ± 3.35
Height (cm)	159.72 ± 3.16	160.44 ± 3.54	158.65 ± 3.33	159.53 ± 4.73	159.72 ± 3.16
Weight (kg)	74.53 ± 5.86	77.32 ± 4.97	75.17 ± 5.39	74.84 ± 6.17	74.04 ± 5.86
BMI (kg/m^2)	29.25 ± 2.16	30.08 ± 2.11	29.82 ± 1.47	29.23 ± 2.05	29.28 ± 2.16

Data are shown as mean ± SD. P: Placebo, O: Omega-3 supplement, T: Aerobic training, TO: Aerobic training + Omega-3 supplement groups. BMI: Body mass index.

Study Design

The subjects of the study had not any history of cardiovascular diseases, type 2 diabetes, hypertension and different type of cancers, not participating in regular exercise training in last year, did not consuming nutritional supplements for at least six months before beginning the present study, and had not prescribed blood pressure and circulating lipid lowering medications. It should be noted that the present study was a randomized double-blind placebo-controlled trial that was registered in the Iranian registry of clinical trials (registration code: IRCT20200722048167N1). All conditions, limitations, disadvantages, benefits and side effects of present study interventions were explained to the subjects. Then, all subjects signed informed consent. Subsequently, subjects were randomly divided into four equal groups including: Placebo (N=10), omega-3 supplement (N=10), aerobic training (N=10), and aerobic training + omega-3 supplement (N=10) groups. The study interventions were consisted of aerobic exercise training, omega-3 supplementation or both (aerobic training + omega-3 supplementation), which both of them conducted for eight weeks according to the considered procedure.

Training Program

The aerobic training program conducted for eight weeks and three sessions per week. Aerobic training intensity was 50-55% HRmax in the first two weeks, 55-60% HRmax in the second two weeks, 60-65% HRmax in the third two weeks, and 65-70% HRmax in the last two weeks (17). Each aerobic training session was about 20 minutes. Before and after each exercise session, 10 minutes warm-up and eight min cool down were performed respectively. During eight weeks intervention, the subjects in the control group continued their daily routine life.

Omega-3 Supplementation

Omega-3 supplementation was 2000 mg daily for omega-3 fatty acid supplement and aerobic training + omega-3 supplement groups, which is an approved dose without any side effects for obese women (18). Omega-3 supplement was consumed as two 1000 mg capsules in the morning and night (with or after breakfast and dinner). The placebo group also consumed 2 g oral paraffin oil daily. Omega-3 supplements was purchased from the Karen Company.

Biochemical Measurement

Blood samples collected at the baseline and after completing the 8 weeks intervention

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(training, omega-3 fatty acid supplement, training + omega-3 fatty acid supplement). Collected blood samples were poured into a falcon tube and were centrifuged for 15 minutes at 3000 rpm, and the obtained serum samples feezed in -80 C until the laboratory assessment. The serum levels of Paraoxonase (USCN company, Cat Num: SEA243Hu, Sensitivity: 1.27ng/ml) and Myeloperoxidase (Cusabio company, Cat Num: CSB-E08721h, Sensitivity: 0.39 ng/ml) were measured by ELISA method according to their manufacturer instruction. Apo-A1 and Apo-B levels were analyzed by using immunoturbidimetry and autoanalyzer. In addition, lipid profile (LDL-C, HDL-C) levels were measured by Pars Azmoon kit. Maximal oxygen uptake (VO2max) was analyzed through the Rockport Walk Test (RWT) according to the previous researches (19).

Statistical Analysis

The data of the study were analyzed by SPSS-24 software. Our data had normal distribution (determined by Shapiro-Vilk test). For between groups analysis, the analysis of covariance with Bonferroni post-hoc test was used. In addition, within group differences were determined by paired t test. The significance level was considered at $P < 0.05$ for all analysis steps and if p value was less than 0.05, the changes were considered significant statistically.

Results

Our findings indicated a significant difference between the studied groups for body weight, body fat percent, LDL-C, Apo-A1 and Apo-B ($P < 0.001$), HDL-C ($p=0.005$), and VO_{2max} ($P = 0.005$). Bonferroni post-hoc test indicated that HDL-C level increased significantly in T ($P = 0.012$) and T + TO ($P = 0.025$) groups compared to the P group. On the other hand, LDL-C levels significantly decreased in T and TO groups compared to P and O groups ($P < 0.001$). In addition, significant decrease in BMI and body fat percentage were observed in T and TO groups compared to P and O groups ($P < 0.001$). On the other hand, VO_{2max} significantly increased in T and TO groups compared to P and O groups ($P < 0.001$) (Table2). According to Bonferroni post-hoc test, Apo-A1 levels significantly increased in T compared to P ($P = 0.001$), and also in TO group compared to P ($P < 0.001$) and O ($P = 0.001$) groups. However, there was no significant difference between P and O ($P = 0.149$) groups, and between T and TO ($P = 0.104$) groups. In addition, Bonferroni post-hoc test indicated a significant decrease of Apo-B levels in both T and TO groups compared to P and O groups ($P < 0.05$). Moreover, the observed difference for Apo-B levels between O and P ($P = 0.181$), and T with TO ($P = 0.20$) groups was not statistically significant. There was a significant difference between the groups for serum levels of paraoxonase ($P < 0.001$). Bonferroni post-hoc test indicated that paraoxonase levels significantly increased in O ($P = 0.001$), T ($P = 0.001$), and TO ($P < 0.001$) groups compared to P group. However, there was no significant difference between T and O ($P > 0.001$), TO with T ($P > 0.001$), and TO with O group ($P = 0.848$) (Figure 1).

Analysis of covariance suggested that the between groups differences for the myeloperoxidase levels was statistically significant ($P < 0.001$). Myeloperoxidase levels indicated a significant decrease in the T compared to the P and O groups ($P < 0.001$), and in the TO compared to the P ($P < 0.001$) and O ($P = 0.002$) groups (Figure 2).

Discussion

The present study conducted aimed to determine the effect of eight weeks aerobic training along with omega-3 ingestion on the levels of paraoxonase and myeloperoxidase in the sedentary overweight and obese women. The present study main findings were that, serum levels of paraoxonase in omega-3, training, and

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training + omega-3 groups significantly increased compared to placebo group, and myeloperoxidase significantly decreased in training and training + omega-3 groups compared to placebo and omega-3 groups.

Another finding of present study was that, Apo-A1 significantly increased in training and training + omega-3 groups compared to placebo and omega-3 groups.

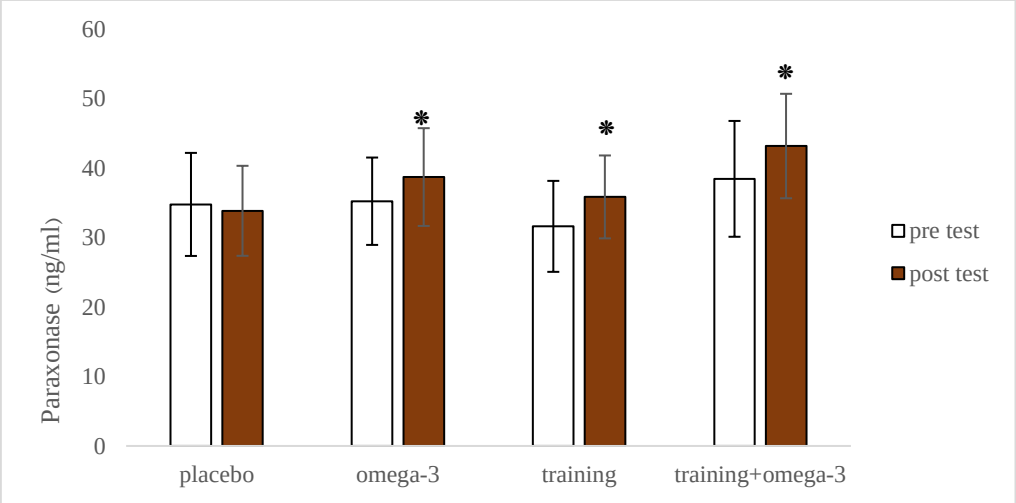

Figure 1. Paraoxonase levels in different groups at pre- and post-test steps of the study. * Significant increase compared to the placebo group.

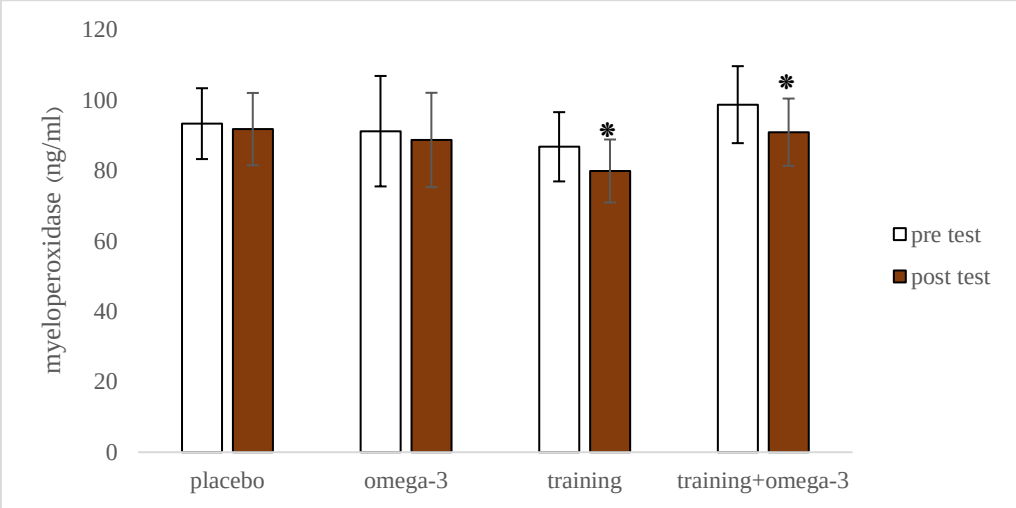

Figure 2. levels in different groups at pre- and post-test steps of the study. * Significant decrease compared to placebo and omega-3 fatty acids supplement groups.

In addition, significant decrease of Apo-B was observed in both trained (training and training + omega-3) groups compared to placebo and omega-3 groups. It is reported that, ApoA1 is one of the major units in the formation of HDL-C and a vital component of HDL-C, which remarkably synthesized in the liver and gastrointestinal tract, and secreted into the plasma, and through act of peripheral tissue membrane proteins (known as ABCA1), cell cholesterol transferred to ApoA1 and HDL-C is formed (20). In addition, ApoB is a protein component of LDL-C, which increases cellular cholesterol uptake by combining LDL components with LDL receptors, and hence, ApoB is associated with LDL-c levels and incidence of cardiovascular

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disease, and is known as a risk factor (21). Previous studies suggested that ApoA1 levels increase following long-term exercise training (22), and the ApoA1 levels upregulation was observed after eight weeks aerobic training in the present study. Consistent with the present study findings, researchers reported that eight weeks aerobic training alone or combined with saffron supplementation in type-2 diabetic men result in increased ApoA1 levels (23). These researchers suggested that changes in the circulating adiponectin and its levels in various tissues may be effective in increasing ApoA1 levels, although changes in the levels of other factors such as LCTA, cholesterol acyltransferase (ACAT) and CEPT following exercise can result in increased the ApoA1 levels (23). Unfortunately, in the present study, changes in the levels of above-mentioned factors have not been investigated.

It seems that different types of exercise training can reduce the ApoB/A1 ratio, as Paoli et al. (2013) reported that 12 weeks of high and low intensity circuit training, and endurance training in overweight middle-aged men leads to a significant decrease in ApoB/A1 ratio, which was accompanied by decrease in LDL-c and cholesterol in all training groups, and increase in HDL-c levels in the high intensity circuit training group (24). In another study, showed that 12 weeks aerobic training (50 to 60% VO_{2max}) in obese women significantly decreased ApoB levels, and ApoB/ApoA-1 ratio (25), which in line with the present findings, simultaneous decrease in BMI and an improvement in lipid profile were observed, but ApoA1 levels don't changed significantly, which no change in the ApoA1 levels following aerobic training attributed to insufficient intensity and volume of exerted exercise training (24). On the other hand, the present study findings indicated that omega-3 supplementation alone don't has a significant effect on ApoA1 and ApoB levels, and aerobic training+omega-3 don't exert further effect on ApoA1 and ApoB levels compared to aerobic training alone. Although, there is no information regarding to simultaneous effect of exercise training and omega-3 supplementation on the levels of ApoA1 and ApoB, consistent with the present findings, Wohl et al. (2005) indicated that 4 and 16 weeks of omega-3 supplementation don't have a significant effect on ApoA1 levels (25). Thusgaard et al (2009) investigated the effect of 12 weeks omega-3 supplementation in HIV patients, and showed no significant change in ApoA1 and ApoB levels, which was associated with no significant improvement in lipid profile (26). In another study, Toorang et al (2015) reported no significant change in ApoA1 levels, but significant decrease in ApoB levels after eight weeks omega-3 supplementation in type 2 diabetic patients (27). The contradiction findings regarding to ApoB changes compared to present study can be attributed to the difference in subjects' physical characteristics, as well as the higher dose of omega 3 supplement (2700 mg) than present study.

Another finding of present study was that serum levels of paraoxonase in the omega-3, training and training+omega-3 groups significantly increased compared to the placebo group. In addition, a significant decrease in serum myeloperoxidase levels was observed in the training and training + omega-3 groups compared to placebo and omega-3 groups. Myeloperoxidase act on LDL and HDL, and lead to chemical and oxidative changes in lipid and protein content (28). In addition, paraoxonase exert anti-atherogenic and anti-inflammatory effects and reduce HDL exposure to peroxidation (29). Paraoxonase also protects LDL and HDL against oxidative stress (30). Therefore, it seems that myeloperoxidase/ paraoxonase ratio indicates the function of lipoproteins and increasing this ratio can be considered as an important marker for cardiovascular disease, and it's reported that isolated HDL from patients with high

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myeloperoxidase/paraoxonase ratio shows attenuation of anti-inflammatory properties (9). Consistent with our findings, Ghorbanian and Shokrollahi (2017) reported that eight weeks exercise training (roping) in sedentary girls, resulted in a significant increase in paraoxonase levels and improving lipid profile (11).

It seems that, changes in paraoxonase levels can also vary depending on the type of exerted training. In this regard, Mahdirejei et al (2015) observed that four weeks aerobic training in obese men is associated with increased paraoxonase levels, but four weeks resistance training had no significant effect on paraoxonase levels (15). In another study, Casella et al (2011) reported that resting paraoxonase levels in individuals with metabolic syndrome were significantly lower than in healthy controls, indicating decrease in paraoxonase levels in obesity and metabolic syndrome (31), and reported that 12 weeks aerobic training leads to a significant increase in paraoxonase levels in patients with metabolic syndrome (31). Some researchers have also shown that even short-term exercise training can affect paraoxonase. In this regard, Koncsos et al (2011) observed a significant increase in paraoxonase activity after 2 weeks lifestyle intervention (exercise training and diet), and showed a decrease in paraoxonase activity in obese compared to normal weight children (32). These researchers suggested that obesity may reduce the paraoxonase activity by increasing ROS, and its expression is inhibited by increasing the acute phase response (32). In another study, researchers suggested that adiponectin could affect the observed changes in paraoxonase levels, and hepatic paraoxonase production can affected by adiponectin (33). Unfortunately, in the present study, changes in ROS and adiponectin levels were not investigated.

Regarding the effect of exercise training on myeloperoxidase levels, Richter et al (2015) consistent with the present findings indicated that 12 weeks endurance training significantly decreased the myeloperoxidase levels in overweight subjects, and researchers linked the myeloperoxidase downregulation to the anti-atherosclerotic and cardioprotective effects of exercise training (34). However, contrary to the present findings, Alishahi et al (2019) reported that 10 weeks of aerobic training alone and in combination with vitamin C supplementation in hypertensive overweight men did not have a significant effect on myeloperoxidase levels, and only a slight decrease in myeloperoxidase levels was observed (35), the pathological conditions of above-mentioned study subjects (hypertensive against healthy overweight and obese subjects in present study) could explain contradictory with the present findings.

Despite the above-mentioned findings, we observed that omega-3 supplementation alone results in significant increase in paraoxonase levels, but we don't indicate any change in myeloperoxidase levels following omega-3 supplementation. In addition, although the increase in paraoxonase and decrease in myeloperoxidase levels was further in the omega-3+training group compared to other groups, but no significant difference was observed between the training and training+omega-3 groups, and omega-3 supplementation could not exert a synergistic effect combined with aerobic training. Kotur et al (2015) confirmed present study findings and suggested that eight weeks omega-3 ingestion, increased paraoxonase activity in middle-aged dyslipidemic subjects (36). In addition, Schiano et al (2008) consistent with the present findings reported that 12 weeks omega-3 supplementation in patients with peripheral vascular disease has no significant effect on myeloperoxidase levels (12). However, due to limited conducted studies, especially regarding the effect of omega-3 supplementation alone or in combination with exercise training on the levels of paraoxonase and myeloperoxidase, on the other hand,

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because of present study limitation including little investigated sample size, short term period of intervention and, inability to accurately control of subjects' nutrition, identifying the effect size and mechanism of aerobic training and omega-3 supplementation for modulating the levels of paraoxonase and myeloperoxidase, needs further investigation and should be considered in the future studies.

Conclusion

It seems that, despite exercise training and omega-3 supplementation roles in modulating the paraoxonase and myeloperoxidase levels, as well as improving the lipid profile, these interventions (exercise training and omega-3) simultaneously does not have a further impact on studied variables. Based on the available evidence, it can be concluded that lack of significant differences between training and training + omega-3 groups for observed changes in paraoxonase and myeloperoxidase in the present study can be attributed to the short duration (eight weeks) of intervention.

Acknowledgments

This article written according to findings of exercise physiology Ph. D thesis and was approved by the ethics committee of Islamic Azad University- Science and Research Branch with the following ethical code: IR.IAU.SRB.REC.1399.010.

Conflict of Interest

The authors declare that no conflict of interest exists.

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