Effects of aerobic training and omega-3 supplementation on the levels of CRP, MDA and lipid profile in overweight and obese women

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Abstract

Introduction: Exercise training and omega 3 supplements play an important role in modulating the levels of oxidative and inflammatory mediators. The present study conducted aimed to investigate the effects of aerobic training and omega-3 supplementation on the levels of oxidative (MDA) and inflammatory (CRP) mediators and lipid profile in overweight and obese women.
Materials and Methods: The 40 overweight and obese women (BMI = 29.1 ± 6.9 kg/m^2) randomly assigned to four groups (10 subjects in each group) including placebo (C), omega-3 (S), training (T) and training + omega 3 (ST) groups. Aerobic training program was performed with 50-70 percent of maximum heart rate for eight weeks/three sessions per week. Daily 2000 mg omega 3 supplement were also consumed by S and ST groups. The MDA, CRP and lipid profile levels were measured and data were analyzed by SPSS software (version 24).
Results: Significant decrease in CRP levels in S ($P = 0.014$), T ($P = 0.030$) and ST ($P < 0.001$) groups compared to C group, and significant decrease in MDA levels in T and ST groups compared to C and S groups were observed ($P < 0.05$). In addition, lipid profile indicated a significant improvement in T and ST groups ($P < 0.05$).
Conclusion: Despite the aerobic training and training + omega-3 supplement effects in decreasing levels of CRP, MDA and lipid profile improvement, no significant difference was observed between the two groups. It seems that, eight weeks omega-3 ingestion along with aerobic training don't exert a synergistic effect.

Keywords: Aerobic training, Inflammation, Oxidative stress, Omega-3

Introduction

Due to consumption of high calorie diets and sedentary lifestyle, the obesity incidence has remarkably increased in past decade in developed and developing countries, as more than a third of the world's adults are overweight or obese (1). Every year, 28 million people die worldwide due to obesity and overweight related disorders, such as hypertension, dyslipidemia, insulin resistance, stroke, diabetes mellitus, fatty liver disease, coronary artery disease, cancer, and metabolic diseases (2). Obesity-related disorders are mainly associated with inflammation (3) and obesity considered as a low-grade inflammatory condition (4), which characterized by an increase in the number and activation of immune cells such as macrophages, neutrophils, and T helper cells, which in turn cause the

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secretion of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukins (ILs) and C-reactive protein (CRP), and simultaneously suppress anti-inflammatory cytokines (5). CRP is a well-known inflammatory risk factors which has attracted a lot of attention and its high levels are associated with increased prevalence of cardiovascular diseases (6).

In addition to inflammation, obesity may be a state of chronic oxidative stress, which considered as a possible mechanism underlying the development of co-morbidities in obesity (7). Oxidative stress exerts different pathological effects, and over-expression of oxidative stress damages cellular structures along with down-regulation of anti-oxidant mechanisms, result in development of obesity-related complications (8). Malondialdehyde (MDA) considered as a main oxidative stress marker in obese subject's and MDA levels is significantly higher in obese subjects compared to non-obese healthy controls, which represented that obesity is an independent risk factor for plasma lipid peroxidation (9). It's reported that, MDA has a positive correlation with body mass index (BMI), which suggested that obesity play an important role in MDA upregulation (10). Therefore, MDA measurements in blood or tissue homogenates is an effective procedure to predict the oxidative stress levels (11).

Despite the pathological effect of obesity and overweight, there is a different pharmacological and non-pharmacological method for obesity managements (12). Among non-pharmacological strategy, endurance training attracted a lot of attention because of its role in combating obesity related disorders, increase lipid oxidation and insulin sensitivity (13). Exercise training are known to exert anti-inflammatory effects by different mechanism (14). Moreover, its reported that exercise training and regular physical activity is associated with reduced oxidative stress and exercise training is an important antioxidant strategy (15). In addition to exercise training, its suggested that omega-3 supplement have potential anti-inflammatory activity in a variety of inflammatory condition (16). Similar to exercise training, omega-3 supplementation attenuates oxidative stress related damages (17). In fact, exercise training and omega-3 supplement both has an anti-inflammatory and antioxidant effects. Regarding the simultaneous effect of exercise training and omega-3 supplement, enhanced cardiometabolic benefits of aerobic training in metabolic syndrome patients along with omega-3 supplementation have been reported (18). In contrast, some researchers suggested that resistance training along with omega-3 supplementation does not improve body composition or lower inflammatory markers more than resistance training alone (19). Generally, the results about exercise training effects combined with omega-3 supplementation on inflammatory and oxidative stress factors are limited and contradictory. Therefore, present study conducted aimed to investigate the effects of eight weeks aerobic training and omega-3 supplementation on the levels of CRP, MDA and lipid profile in overweight and obese women.

Materials and Methods

Participants

Overweight and obese women age ranging 25 to 40 years old comprised the present study subjects, which among recruited volunteer subjects, 40 overweight and obese women selected to take part in present study protocol. Inclusion criteria for present study subjects were as follows: Age 25-40 years old, no history of cardiovascular diseases, type 2 diabetes, hypertension and different type of cancers and malignancies, not participating in regular exercise training in last year, not consumption blood pressure and circulating lipid lowering medications,

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don't take nutritional supplements for at least six months before beginning the present study. Exclusion Criteria consist of don't take part in blood sampling session (pretest or posttest), simultaneous participation in other exercise training or regular physical activity program, no regular participation in designed exercise training program, physician advice for interrupt or terminate training program or omega-3 supplementation, incidence of disease during intervention, subjects' injuries during training program. The present study was conducted according to the principals of the Declaration of Helsinki and the study protocol was approved by the ethics committee of Islamic Azad University- Science and Research Branch (Ethical codes: IR.IAU.SRB.REC.1399.010).

Study Design

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 including aerobic training, omega-3 supplementation or their combination (aerobic training+ omega-3 supplement) are explained to the subjects. Ethical principles were considered at all stages of study, and subjects could withdraw from the study whenever they want. Then, subjects were still willing to participate in the present research, signed informed consent. Subsequently, subjects were randomly classified into four equal groups (10 subjects in each group) including: 1. Placebo (not participating in aerobic training program, not taking omega-3 supplements). 2. Omega-3 supplement (taking omega-3 supplement, not participating in aerobic training program). 3. Aerobic training (participation in aerobic training program, not taking omega 3 supplements), and aerobic training+omega-3 supplement (take omega-3 supplement along with participation in aerobic training program) groups.

Intervention

Present study interventions consist of aerobic exercise training, omega-3 supplementation or both (aerobic training+omega-3 supplementation), which both of them conducted for eight weeks according to considered procedure.

Aerobic 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 (20). 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 considered 2000 mg daily for omega-3 and aerobic training+omega-3 supplement groups, which is an approved dose without any side effects for obese women (21). Omega-3 supplement is 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 Karen Company.

Blood Sampling and Laboratory Assessment

Blood samples collected at the baseline and after completing the 8 weeks intervention (training, omega-3, training+omega-3). Collected blood samples were poured into a falcon tube

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and were centrifuged for 15 minutes at 3000 rpm, and obtained serum samples freeze in -80 C until laboratory assessment. Serum MDA levels were determined using the Draper and Hadley methods based on thiobarbituric acid (TBA) reactivity (22). Serum CRP levels were also measured by Pars Azmoon diagnostic kit. In addition, lipid profile (cholesterol, triglyceride, LDL-c, HDL-C) levels were measured by Pars Azmoon kit. In order to measure the body fat percentage, a body composition analyzer (BOCA-X1) was used.

Statistical Analysis

Present study data were analyzed by SPSS (version 24) software. First, data distribution determined by Shapiro-Vilk test, which represented a normal data distribution ($P > 0.05$). In order to between groups analysis, the analysis of covariance test with Bonferroni post-hoc test was used. In addition, within group difference were determined by paired t test. The significance 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

Subjects' characteristics including age, height, body weight and BMI in the different groups are represented in Table 1.

Table1. Subjects' characteristics in the study.

Variable	Placebo	Omega-3	Aerobic Training	Aerobic Training + Omega-3
Age (years)	28.3 ± 3.63	26.7 ± 3.19	26.2 ± 2.98	27.8 ± 3.35
Height (cm)	159.8 ± 4.73	158.6 ± 3.33	160.4 ± 3.54	159.7 ± 3.16
Weight (kg)	74.8 ± 6.17	75.1 ± 5.39	77.3 ± 4.97	74.5 ± 5.86
BMI (kg/m^2)	29.2 ± 2.05	29.8 ± 1.47	30.0 ± 2.11	29.2 ± 2.16

Data are shown as Mean ± SD.

Analysis of covariance test indicated a significant difference between groups for BMI, percent body fat, HOMA, cholesterol, triglyceride, LDL-c ($P < 0.001$), and HDL-c ($P = 0.005$) levels. According to Bonferroni post-hoc test results for BMI and percent body fat, there was a significant difference between training and training+omega-3 groups with placebo and omega-3 groups ($P < 0.001$). The HOMA findings represented a significant difference between training with placebo ($P = 0.012$), and training + omega-3 with placebo ($P = 0.001$) and omega-3 ($P = 0.009$) groups. In addition, a significant difference between training group with placebo ($P = 0.011$), and training + omega-3 with placebo ($P = 0.001$) and omega-3 ($P = 0.004$) groups for cholesterol levels were observed (Table 2). Bonferroni post-hoc test for triglyceride levels indicated a significant difference between training with placebo ($P < 0.001$) and omega-3 ($P = 0.041$) groups, and training+omega-3 with placebo ($P < 0.001$) and omega-3 ($P = 0.004$) groups. Moreover, significant difference between training and training + omega-3 with placebo and omega-3 groups were observed for LDL-c levels ($P < 0.001$). The HDL-c levels data analysis, indicated a significant difference between training ($P = 0.012$) and training+omega-3 ($P = 0.025$) groups with placebo group (Table 2).

Intragroup analysis with paired t test indicated a significant decrease of BMI, percent body fat and HOMA in training and training+omega-3 groups ($P < 0.001$). In addition, cholesterol and triglyceride levels significantly decreased in omega-3, training and training + omega-3 groups ($P < 0.05$), and significant decrease of LDL-c levels were observed in training and training+ omega-3 groups ($P < 0.001$). Moreover, HDL-c significantly increased in training ($P = 0.014$) and training+ omega-3 ($P = 0.013$) groups.

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Between group CRP analysis indicated a significant difference (P < 0.001). The significant difference between training (P = 0.030) and training + omega-3 (P < 0.001) groups with placebo group were observed. Paired t test indicated a significant decrease of serum CRP levels in omega-3 (P = 0.005), training (P = 0.001) and training + omega-3 (P = 0.006) groups, but observed change in CRP levels was not significant in the placebo group (P = 0.327).

Table 2. The level of variables determined in different groups of the current study.

Variables	Stage	Placebo	Omega-3	Training	Training + Omega-3	P value
BMI (kg/m^2)	pre test	29.2 ± 2.05	29.8 ± 1.47	30.0 ± 2.11	29.2 ± 2.16	
	post test	29.3 ± 2.02	29.9 ± 1.49	29.5 ± 1.96	28.7 ± 2.05	< 0.001
Paired t test		P = 0.596	P = 0.177	P < 0.001	P < 0.001	
Percent body fat	pre test	31.2 ± 3.17	32.4 ± 2.54	33.1 ± 2.73	31.5 ± 2.97	
	post test	31.6 ± 3.43	32.6 ± 2.32	31.6 ± 2.70	30.1 ± 2.87	< 0.001
Paired t test		P = 0.032	P = 0.256	P < 0.001	P < 0.001	
Glucose (mg/dl)	pre test	91.6 ± 6.85	89.7 ± 7.68	92.8 ± 6.69	92.1 ± 8.08	
	post test	90.8 ± 6.57	88.4 ± 5.46	90.1 ± 6.04	88.9 ± 6.22	0.202
Paired t test		P = 0.280	P = 0.231	P = 0.040	P = 0.004	
Insulin (mU/ml)	pre test	8.6 ± 1.05	8.4 ± 0.84	9.1 ± 0.76	8.7 ± 0.82	
	post test	8.4 ± 0.93	8.3 ± 0.73	8.3 ± 0.86	8.0 ± 0.65	0.001
Paired t test		P = 0.333	P = 0.251	P < 0.001	P < 0.001	
HOMA-IR	pre test	1.95 ± 0.35	1.87 ± 0.27	2.08 ± 0.16	1.99 ± 0.27	
	post test	1.90 ± 0.29	1.81 ± 0.22	1.86±0.22	1.76 ± 0.23	< 0.001
Paired t test		P = 0.158	P = 0.103	P < 0.001	P < 0.001	
Cholesterol **(**mg/dl**)**	pre test	207.9 ± 21.39	224.5 ± 29.17	211.7 ± 25.02	218.2 ± 26.65	
	post test	203.4 ± 19.83	216.1 ± 27.40	197.1 ±2 2.61	199.6 ± 24.31	< 0.001
Paired t test		P = 0.085	P = 0.001	P < 0.001	P < 0.001	
Triglyceride **(**mg/dl**)**	pre test	151.4 ± 11.73	163.9 ± 14.05	148.7 ± 9.22	157.1 ± 12.16	
	post test	153.8 ± 13.26	157.6 ± 9.32	139.5 ± 6.51	144.3 ± 9.52	< 0.001
Paired t test		P = 0.295	P = 0.008	P < 0.001	P < 0.001	
LDL-c (mg/dl)	pre test	166.7 ± 11.40	161.3 ± 12.65	157.5 ± 10.98	171.2 ± 15.76	
	post test	172.1 ± 12.35	163.7 ± 10.84	151.4 ± 9.44	159.7 ± 13.68	0.005
Paired t test		P = 0.023	P = 0.351	P < 0.001	P < 0.001	
HDL-c (mg/dl)	pre test	48.3 ± 5.77	46.9 ± 3.84	46.4 ± 4.69	47.8 ± 5.90	
	post test	47.5 ± 4.52	47.1 ± 3.17	48.2 ± 3.58	49.1 ± 5.46	< 0.001
Paired t test		P = 0.259	P = 0.735	P = 0.014	P = 0.013	

Data are shown as Mean ± SD.

In addition, analysis of covariance test noted a significant difference between groups for MDA levels (P < 0.001). According to Bonferroni post hoc test, significant difference between training with placebo and omega-3 groups (P < 0.001), and between training + omega-3 group with placebo (P = 0.015) and omega-3 (P = 0.011) groups were observed. Moreover, paired t test indicated that MDA levels significantly decreased in training (P < 0.001) and training + omega-3 (P = 0.001) groups. However, no significant changes were observed for omega-3 (P = 0.095) and placebo (P = 0.331) groups.

Discussion

In the present study, the effects of aerobic training and omega-3 supplementation on the levels of oxidative (MDA) and inflammatory (CRP) mediators and lipid profile in overweight and obese women have been investigated. Present study main findings were that eight weeks aerobic training alone and combined with omega-3 supplementation result in significant decrease in CRP and MDA levels. Although omega-3 supplementation alone was also associated with significant decrease of CRP levels, there was no significant difference between omega-3

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and placebo groups. In addition, no significant difference was observed between training and training+omega-3 groups for observed changes in CRP and MDA levels. Obesity is a main risk factor for developing the metabolic syndrome and type2 diabetes (23). Among different recommended strategy for obesity management, exercise training and regular physical activity play an important role in weight loss and maintenance, and decreasing obesity related disorders (24).

Consistent with the present findings regarding the exercise training effect in decreasing CRP levels, its reported that exercise training is an anti-inflammatory strategy (25). Mogharnasi et al (26) reported that both endurance and resistance exercise training for eight weeks can decrease the obesity-related factors including CRP, vaspin, and BMI (26). In addition, Ryan et al (27) observed that 6-month aerobic training led to lowering CRP levels, which was associated with significant decrease in BMI, percent body fat and visceral fat (27). However, some researchers suggested that endurance training is more effective in decreasing the CRP levels. For example, comparing the effect of 12 weeks resistance, endurance and combined training in middle aged men indicated a significant decrease of CRP only in endurance training (28), which confirmed the present findings. Therefore, different exercise training type hasn't a similar effect on inflammation. Decrease inflammatory mediators following different type of exercise training exerted by different mechanism, including increasing skeletal muscle mass and adaptation in immune cells (29), and especially decrease adipose tissue recognized as a main mechanism for anti-inflammatory effect of exercise training including decrease CRP levels (30).

Although, its suggested that omega-3 supplement has anti-inflammatory properties like exercise training (16), present study indicated that omega-3 supplementation don't has a significant effect on CRP levels compared to placebo group and exercise training combined with omega-3 ingestion don't magnitude the exercise training effect in decreasing CRP levels. In accordance with the present findings, eight weeks omega-3 consumption in diabetic patients don't changes CRP levels, but omega-3 supplementation was associated with significant decrease of TNF-α and significant increase in IL-2 cytokines (31). These findings represented that anti-inflammatory effect of omega-3 supplementation exert by decreasing other inflammatory (TNF-α) and increasing anti-inflammatory (IL-23) mediators, except CRP levels. In contrast, decrease of CRP levels in hypertensive and diabetic obese adults observed after eight weeks omega-3 ingestion (32). Contradiction with the present findings probably is related to different subject's characteristic compared to present findings. There is a limited information about simultaneous effect of exercise training and omega-3 supplementation on inflammatory mediators. Present findings confirmed by previous studies and Cornish et al (19) indicated that despite decrease inflammation in training and training+omega-3 groups, omega-3 supplementation along with resistance training does not improve body composition or lower inflammatory factors (IL-6, TNF-α) more than resistance training alone in older men (19). In another study, researchers observed that TNF-α and IL-6 levels changes in post-menopausal women (as inflammatory cytokines) after 12 weeks aerobic training alone or with omega-3 supplementation wasn't significant between training g and training+omega-3 groups, but these researchers suggested that 24 weeks aerobic training along with omega-3 supplementation cause a further decrease in TNF-α and IL-6 levels compared to exercise training group (33). According to above mentioned results, it seems that long term omega-3 supplementation causes a

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significantly further improvement in inflammatory pathways following exercise training.

Our findings about exercise training effect in decreasing MDA levels are supported by those of a previous study who reported that eight weeks aerobic training in middle-aged men remarkably decreased MDA levels (34). In addition, investigate the effect of aerobic, resistance and combined training in untrained males indicated a significant decrease of MDA in all trained groups, which simultaneously increased in antioxidant enzyme (GPx and total antioxidant capacity) were observed (35). According to these findings, exercise training recognized as important antioxidant, which attenuated the oxidative stress (36). Consistent with our study, Pooya et al (37) reported that eight weeks omega-3 consumption don't has a significant effect on MDA levels in type 2 diabetic patients compared to placebo group (37). Moreover, present findings clearly suggested that 8 weeks omega-3 supplementation with aerobic training cannot maximize the exercise training effect in decreasing MDA levels. A potential mechanism by which these protective benefits of exercise training in decrease MDA levels and lowering oxidative stress may occur are through attenuated the expression of oxidative stress in white adipose tissue (38). In addition, decrease adipose tissue and obesity in trained groups can be potential mechanism for decreasing oxidative stress and MDA levels, as reported that obesity per se can induce systemic oxidative stress through various biochemical mechanisms, including superoxide generation from NADPH oxidases, glyceraldehyde auto-oxidation, oxidative phosphorylation, protein kinase C activation, and polyol and hexosamine pathways (39).

Other findings of present study were that cholesterol levels in training compared to placebo, and in training+omega-3 group compared to placebo and omega-3 groups decreased significantly. In addition, significant decrease of triglyceride and LDL-c in training and training+omega-3 groups compared to placebo and omega-3 groups were observed. Moreover, HDL-c significantly increased in training and training+omega-3 groups compared to placebo and omega-3 groups. In fact, eight weeks omega-3 ingestion don't enhance the exercise training effect in improvement the lipid profile. Researchers consistent with present findings observed that eight weeks combined (endurance-resistance) training and omega-3 supplementation was associated with improvement lipid profile (cholesterol, TG, LDL-c), but no significant difference were observed between two trained groups and omega-3 don't exert a synergic effect (21). However, in contrast to present findings, Ortega et al (18) reported that supplementation with omega-3 fatty acids and oleate enhances exercise training effects in decreasing the levels of TG and increase the HDL-c levels in patients with metabolic syndrome, Contradiction findings with the present study probably is related to long term (24 weeks) intervention period compared to present study (18).

Generally, exercise training known as an effective intervention for lipid profile improvement (40). Although, the exact mechanism by which exercise training affects circulation lipids is still unknown, exercise training can increase lipid intake and thus lead to decreased lipid levels (41). In addition, exercise training increased the lipoprotein lipase (LPL) activity, which is responsible for the hydrolysis of chylomicrons and VLDL in granules (42). In support of this hypothesis, an increase in LPL levels and activity with aerobic training has been observed (43). However, respect to few information especially about the simultaneous effect of exercise training and omega-3 supplementation, their effectiveness mechanisms including in reducing inflammation and oxidative

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stress, should be further investigated in future studies.

Conclusion

In conclusion, it seems that despite positive effect of exercise training and omega-3 ingestion including improvement the inflammatory and oxidative stress related factors and also improving lipid profile, this intervention simultaneously can't exert further effect on inflammation and oxidative stress. According to existence evidence, it can be concluded that increasing the intervention duration probably was associated with further changes, and no significant difference between training and training+omega-3 groups in the present study can be attributed to short (8 weeks) intervention period.

Conflict of interest

The authors declare that no conflict of interest exists.

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