

Research Paper: The Effects of Two Different Types of Resistance Training Exercise Plus Omega-3-6-9 Supplement on E-selectin and ICAM-1 Adhesion Molecules in Young Overweight Men



Keyvan Ahmadi Dehrashid¹, Marefat Siahkohian^{1*}, Salahadin Ahmadi², Lotfali Bolboli¹

1. Department of Physical Education and Sport Sciences, University of Mohaghegh Ardabili, Ardabil, Iran.

2. Cellular and Molecular Research Center; Research Institute for Health Development, Kurdistan University of Medical Sciences; and the Department of Medical Physiology and Pharmacology, Faculty of Medicine, Kurdistan University of Medical Sciences Sanandaj, Iran.



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ABSTRACT

Background: Performing regular exercise training and taking inflammation-reducing supplements can be efficient in lowering atherosclerosis risk. The aim of this research was to assess the impact of resistance training (RT) conducted with or without Omega3-6-9 (Omega 3) supplementation in ascending pyramid (AP) or circuit (CR) on plasma levels of E-Selectin and ICAM-1 adhesive molecules in young overweight males.

Methods: Sixty healthy overweight men (18-26 years of age) were recruited and randomly allocated to either receive Omega 3 supplement (SU, 2.4 g/d) or placebo (PL, 2% dextrose) for 12 weeks. Topics of SU and PL divided into groups of AP, CR, RT, and Control (C). Levels of plasma E-selectin and ICAM-1 were evaluated and compared before and after the practice training period. ICAM-1 Levels did not change significantly after exercise in any of the groups.

Results: E-selectin levels decreased significantly in CR plus SU (P=0.014) and AP plus SU (P=0.012). ICAM-1 did not change significantly in any of the groups.

Conclusion: Based on the results of this research, RE plus Omega3-6-9 supplement is efficient in decreasing the amount of E-selectin, but not ICAM-1, regardless of the type of exercise.

* Corresponding Author:

Mehdi Marefat Siahkohian, Prof.

Address: Department of Medical Physiology and Pharmacology, Faculty of Medicine, Kurdistan University of Medical Sciences Sanandaj, Iran.

Phone: +98 (914) 4511435

E-mail: M_siahkohian@uma.ac.ir

Introduction

Overweight and obesity, significant risk variables for cardiovascular disease [1, 2], are correlated with sedentary lifestyle. Atherosclerosis, a vascular disease that gradually narrows the inside of an artery due to the accumulation of fat and low-density lipoproteins and the formation of fibrous fat plaques, threatens the health of millions around the world [3, 4]. Recent studies have shown that adhesion molecules, a major player in atherosclerosis pathogenesis, are more sensitive and accurate to predict cardiovascular disease (CVD) risk than lipid profiles [5, 6]. It is known that endothelial dysfunction is involved in atherosclerosis pathogenesis through multiple processes such as enhancing the expression of adhesion molecules [1]. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are part of the immunoglobulin superfamily, which promote the formation of foam cells by binding to monocytes and moving them down into the subendothelial space [7].

ICAM-1 expression at endothelial cells is induced by proinflammatory cytokines such as IL-6 and TNF- α [8]. E-selectin is a cell-surface glycoprotein of the selectin family caused by proinflammatory cytokines such as C-Reactive Protein (CRP) after stimulation of endothelial cells. E-selectin mediates the endothelium wall binding of circulating leukocytes and is involved in inflammation and atherosclerosis [9, 10, and 11]. Endothelial adhesion molecules play important roles in the growth of atherosclerotic plaques and ICAM-1 and E-selectin plasma levels as molecular markers for the prediction of atherosclerosis and CVD development [12]. Studies have shown that resistance training (RT) at low to moderate intensity, 70–75% of one repetition maximum (1RM) in normal and overweight males has no significant impact on cell adhesion molecules reduction [13]. It was noted that performing 3 sets of 8 to 10 repeats low to moderate intensity RT for one year did not alter the concentrations of ICAM-1 and E-Selectin in females [14]. It was shown that exercise-induced muscle damage using a bout of uncommon high-intensity eccentric exercise did not alter the levels of ICAM-1, VCAM-1, and E-Selectin adhesive molecules, but decreased P-selectin adhesion molecule after exercise between 24 and 144 hours [15]. In a study by Koh, Y., et al. (2017), ICAM-1 and VCAM-1 levels did not alter by performing 4 weeks of exercise in overweight males [16]. Some studies have asserted that RT has desired impacts on adhesion molecules [17, 18] as opposed to those results. Hjerkin, E.M., et al. (2005) noted that long-chain n-3 fatty acid supplements in

males with long-standing hyperlipidemia reduced circulating markers of endothelial activation [19]. A lower risk of heart disease was associated with regular exercise programs and anti-inflammatory supplements [13, 20, and 21]. EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) of Omega 3-6-9 (omega 3) prevent thromboxane A₂ production, a vasoconstrictor and platelet aggregator, and are efficient in decreasing CVD threat [21]. Omega 3 can also protect against exercise-induced apoptosis [22]. Resistance exercise (RE) can be a main mediator of better metabolic control through increase in muscle mass [23]. Different RT protocols may affect on inflammatory markers differently. The aim of this research was to determine the effectiveness of 12 weeks of RT performance with 2 distinct loading methods plus Omega 3 supplementation on ICAM-1 and E-Selectin adhesive molecules and to determine whether it could be a suitable intervention to decrease the risk of CVD.

Methods

The present research is quasi-experimental. All youthful overweight males ($25 \leq \text{BMI} \leq 30 \text{ Kg/m}^2$) were the study population. The sample size for a clinical trial was predicted to be 60 people using the standard equation. Subjects, between the ages of 18 and 26, were recruited from masculine learners at Hamedan Payame Noor University who had no regular exercise training in the previous year. The written consent form and Beck Health Questionnaire were completed by the participants. Inclusion criteria include overweight (BMI 25-30 Kg / m²), absence of history of smoking, regular last year workout, CVD family history, acute or chronic diseases. Exit criteria include the inability to follow a diet, the patient's reluctance to continue research or non-compliance with the training protocol given. Subjects were divided into 6 groups of 10 subjects, including ascending pyramid (AP) and circuit resistance (CR) plus Omega3 supplement (SU), CR plus placebo (PL), AP plus PL, SU, and PL. Due to the likely loss of subjects during the workout owing to withdrawal or failure to engage in the research, the groups were first chosen as 12 people and 10 subjects were chosen in each group at the end. During a session, the respondents received to know the working protocol and the right implementation of the movements. A Sartorius digital scale and stadiometer was used to measure the weight and height of the subjects. Training groups performed exercise training for 12 weeks and 3 sessions per week (including: leg extensions, leg curl machine, barbell bench press, back-neck lat pulldown, barbell shoulder press with smith machine, cable cross-over, barbell curl, triceps pushdown, shin exercise and

bench sit-ups) [18, 24]. The experiment group practice schedule consisted of 12 weeks, three weekly sessions, and one hour per session. The subjects warmed up 15 minutes before the workout and cool-down 10 minutes at the end of the session. The subjects had to perform 3 complete rounds of movements in the CR group, with three minutes of rest between each round. In each round, subjects finished 10 stations and repeated them after a 3-minute rest. The first round consisted of 20 repetitions at 40% 1RM, the second round of 15 repetitions at 50% 1RM and the third round of 10 repetitions at 60% 1RM. The subjects had to finish 3 sets of each station in the AP group and then move on to the next station. The rest was 1 and 3 minutes, respectively, between sets and stations. The first set was 6 repetitions at 80 percent 1RM at each station. The second set consisted of 5 repetitions at 85% 1RM and the final set consisted of 4 repetitions at 90% 1RM as previously reported [24]. Throughout the duration of the studies, subjects in CR plus SU, AP plus SU and SU groups got 2.4 gr/day Omega 3-6-9 capsules. In a gelatinous capsule such as Omega3 capsules, CR plus PL, AP plus PL and PL groups got 2 percent dextrose. At the end of each month, in accordance with the gradual overload principle, 1RM of subjects was measured based on predictive equations to estimate 1RM from repetition to fatigue ($1RM (kg) = RepWt (kg) / (1 - 0.02 RTF)$) and the load was determined accordingly for each subject [25]. All participants had a standard diet and during the process of research did not take any medication. Subjects in the PL and SU groups did not

perform any exercise during the study period. Pre and post-test blood samples were obtained from an antecubital vein (10 ml) using the vacutainer system in Farzan laboratory in Hamadan. After the last training session, post-exercise blood samples were drawn 24 hours, while subjects were fast for 12 hours. The laboratory room temperature ranged from 20-25 °C. The levels of ICAM-1 and E-Selectin were measured using ICAM-1 (CK-E10163) and Human E-Selectin ELISA kit (CK-E10176) EASTBIOPHARM, by Enzyme-Linked ImmunoSorbant Assay (COBAS INTEGRA ® 400 plus analyzer, Roche). The ICAM-1 and E-Selectin kits were respectively 9.98 and 51.2 ng/L sensitive. SPSS software version 23 analyzed data. With regarding to the random distribution of subjects in groups and the normality of the data (tested with skewness, kurtosis, and Shapiro-Wilk test), a paired “T” test was used to compare the pre-test and post-test levels of variables between groups. Since all assumptions (normality, variance homogeneity and random independent samples) were met, the ANCOVA test was used to compare the mean CR plus SU and AP plus SU groups after the experiment. Two-way ANOVA was used to determine the impacts of exercise type, supplements, and interaction between exercise-supplements. The amount of significance was 0.05 ($\alpha = 0.05$).

Results

Table 1 shows the demographic information of the subjects.

Table 1. Demographic characteristics of subjects

Groups n=10	Variable					
	Weight (Kg) M ± SD		Height (cm) M ± SD	Age (Year) M ± SD	BMI (kg/m ²) M ± SD	
	Pre-test	Post-test			Pre-test	Post-test
CR + SU	85.13±6.1	82.87±4.1	179.6±6.9	22.7±1.5	26.49±1.31	25.74±1.69
AP + SU	86.8±5.6	85±4.0	180.5±5.0	21.9±1.8	26.63±1.03	26.2±2.43
CR + PL	82.9±4.8	80.6±4.7	174.7±6.6	21.3±1.5	27.24±1.55	26.5±2.05
AP + PL	85.1±6.8	84.5±6.2	177.8±6.2	21.5±1.9	26.94±1.66	26.73±1.59
SU	84.7±5.8	84±5.8	178.7±6.9	21.3±1.6	26.53±1.25	26.62±1.5
PL	81.6±6.5	81.6±6.6	175.9±6.3	21.2±1.9	26.35±0.63	26.38±1.34

CR= Circuit Resistance Exercise, AP: Ascending pyramid resistance exercise, SU= Omega3-6-9 supplement, PL=Placebo

Table 2 shows the results of the pre-test and post-test levels of molecules ICAM-1 and E-Selectin. The mean level of ICAM-1 in the post-test did not differ from the pre-test. In training groups, particularly in CR groups, ICAM-1 values slightly reduced but were not statistically significant. The levels of E-Selectin in CR plus SU (P=0.014) and AP plus SU (P=0.012) groups

decreased significantly. The tests of Shapiro-Wilk, skewed and kurtosis were used to assess the normality of the variables' data distribution.

As shown in Table 3, the values of skewness and kurtosis are within acceptable range (between -2 and+ 2) and the Shapiro-Wilk test findings are higher than 0.05,

Table 2. Mean and standard deviation of adhesive molecules ICAM-1 and E-Selectin in different groups

Groups			Mean	SD	Significant level	
CR + SU n=10	ICAM-1 (ng/L)	Pre-test	1854.70	468.581	0.508	
		post-test	1684.80	556.974		
	E-SELECTIN (ng/L)	Pre-test	1103.80	178.604		0.014*
		post-test	847.0	159.994		
AP + SU n=10	ICAM-1 (ng/L)	Pre-test	1791.60	277.836	0.889	
		post-test	1747.80	747.898		
	E-SELECTIN (ng/L)	Pre-test	1086.80	125.200		0.012*
		post-test	862.70	239.201		
CR + PL n=10	ICAM-1 (ng/L)	Pre-test	2026.0	579.478	0.343	
		post-test	1755.40	775.250		
	E-SELECTIN (ng/L)	Pre-test	1064.70	150.855		0.413
		post-test	978.80	197.169		
AP + PL n=10	ICAM-1 (ng/L)	Pre-test	1908.60	647.187	0.893	
		post-test	1861.10	965.846		
	E-SELECTIN (ng/L)	Pre-test	1072.00	213.359		0.708
		post-test	1023.10	203.748		
SU n=10	ICAM-1 (ng/L)	Pre-test	1856.30	541.991	0.920	
		post-test	1828.40	707.358		
	E-SELECTIN (ng/L)	Pre-test	1027.90	210.230		0.690
		post-test	1069.30	179.649		
PL n=10	ICAM-1 (ng/L)	Pre-test	1976.20	282.760	0.400	
		post-test	1852.10	285.207		
	E-SELECTIN (ng/L)	Pre-test	1096.10	228.645		0.494
		post-test	1036.70	200.425		

CR= Circuit Resistance Exercise, AP: Ascending pyramid resistance exercise, SU= Omega3-6 -9 supplement, PL=Placebo
*P≥0.05

indicating that the variables E-Selectin and ICAM-1 are normally distributed. Levene’s test confirmed the homogeneity of variances.

The resulting p-values for ICAM-1 (P≤0.103) and E-Selectin (P≤0.890) show that the assumption of variance homogeneity is met.

Table 3. Results of skewness and kurtosis tests

		Skewness	Kurtosis	Shapiro-Wilk Significant level
E-Selectin(ng/L)	Pre-test	-0.253	0.301	0.561
	Post-test	-0.044	0.345	0.293
ICAM-1(ng/L)	Pre-test	0.155	0.240	0.130
	Post-test	0.530	0.538	0.132

*Significant difference at p≤0.05

Table 4 presents the results of the covariance assessment of the studied indices. The findings of the regression line slope homogeneity test for ICAM-1 (P 0.332) and E-Selectin (P 0.286) show that the slope assumption homogeneity is met. Based on the p-value acquired for the ICAM-1 ($P \leq 0.910$) and E-Selectin ($P \leq 0.075$) indices, the difference between the post-test

means of two types of exercise trainings at a confidence level of 95 percent is not significant, suggesting that the sort of exercise trainings do not influence the efficiency of exercise on ICAM-1 and E-Selectin factors. Bonferroni post-hoc tests showed no significant difference between groups for ICAM-1 and E-Selectin levels.

Table 4. Results of the covariance analysis of the ICAM-1 and E-Selectin indices

Source	DF	F	Significant level	Eta-squared (η^2)	Test Power
ICAM-1 (ng/L)	5	0.301	0.91	0.030	0.249
E-Selectin (ng/L)	5	2.152	0.075	0.183	0.657

*Significant difference at $p \leq 0.05$

The two-way ANOVA test was used to compare the effects of the type of exercise, supplement, and supplement-exercise interaction on ICAM-1 and E-Selectin indices, (Table 5). For ICAM-1, there was no significant difference between exercise types ($P \leq 0.859$), sup-

plement ($P \leq 0.705$) and supplement-exercise interaction ($P \leq 0.980$). Regarding to E-Selectin, there was no significant difference between exercise type ($P \leq 0.76$), supplement ($P \leq 0.085$) and exercise-supplement interaction ($P \leq 0.237$).

Table 5. Results of two-way ANOVA test for ICAM-1 and E-Selectin of subjects

Source	Type III Sum of Squares	DF	Mean square	F	Significant level	Eta-squared (η^2)	Test Power	
ICAM-1 (ng/L)	Exercise Type	152217.233	2	76108.617	0.153	0.859	0.006	0.195
	Supplement	71829.600	1	71829.600	0.144	0.705	0.003	0.152
	Exercise-Supplement	20085.100	2	10042.550	0.020	0.980	0.001	0.101
E-Selectin (ng/L)	Exercise Type	212669.233	2	106330.067	2.707	0.76	0.91	0.514
	Supplement	121140.267	1	121140.267	3.084	0.085	0.054	0.407
	Exercise-Supplement	116210.533	2	58105.267	1.479	0.237	0.052	0.302

*Significant difference at $p \leq 0.05$

Discussion

Our findings regarding the impact of RT and Omega3-6-9 supplementing on ICAM-1 levels showed that 12 weeks of CR or AP resistance training plus Omega3-6-9 supplement did not change the level of ICAM-1 significantly. Compared to pre-test, the levels of this factor in CR plus PL, AP plus PL, SU, and PL groups did not change significantly in post-test. The ANCOVA and Bonferroni tests showed no significant difference in the reduction of ICAM-1 concentrations between two types of RE. The findings of the two-way ANOVA showed that there was no significant difference between groups with respect to the type of exercise, supplementation, and exercise-supplements interaction. Based on these data, it can be concluded that there is no difference in the level of ICAM-1 between the effects of CR or AP. Montrezol et al. (2014) noted that resistance training in elderly patients with hypertension significantly decreased levels of ICAM-1 and VCAM-1 compared to pre-testing [18]. Sattarzadeh, L., et al. (2017)

found that upper and lower body resistance training in healthy untrained females did not alter the level of ICAM-1 [26]. Factors such as age, gender, type of exercise, exercise duration, sleep disorders and smoking may have led to the discrepancy between our results and what other researchers report. The subjects in present study were healthy young males, while in most of those researches that after exercise there were significant changes in ICAM-1 levels, subjects were older, more obese, or hypertensive. We investigated the impact of RE on ICAM-1 adhesion molecule in the present study, while aerobic exercises were used as the intervention method in most studies that found important changes in levels of ICAM-1. Previous researches showed that the increase in HDL levels results in a reduction in the levels of ICAM-1 and E-Selectin [27]. Since aerobic exercises have a greater impact on the lipid profile, the absence of change in ICAM-1 levels in this research may be due to the absence of change in the lipid profile of subjects that was not measured. Cigarette smoke condensate through protein kinase C activation in endothe-

lial cells increases cell adhesion molecules surface expression including ICAM-1 [28]. Non-smokers were the subjects in the present research, which may contribute to weak response of ICAM-1 to exercise. Our findings showed that E-Selectin levels are significantly reduced by 12 weeks of resistance exercise followed by Omega-3 supplementation in both CR and AP fashions. No significant difference between CR and AP methods was found in the statistical analysis of covariance and Bonferroni post-hoc test. For the type of exercise, supplement and exercise-supplement interaction, the findings of the two-way ANOVA showed no significant difference between the means. Based on the results of this research, it can be indicated that there is no difference in decreasing E-Selectin levels between CR and AP exercises. In combination with Omega3 supplementation, both CR and AP types of exercise can produce beneficial results. Fondong, M., et al. (2016) revealed that in young, sedentary obese male volunteers, E-Selectin levels decreased significantly one hour after a single bout of cycling workout [29]. Saetre, T., et al. (2011) revealed that in middle-aged patients with peripheral arterial disease, levels of E-selectin and ICAM-1 adhesion molecules decreased significantly after 8 weeks of monitored interval walking to close to maximum claudication pain, whereas levels of VCAM-1 did not change significantly [30]. Hjerkin EM et al. (2005) found that n-3 polyunsaturated fatty acids in elderly males with long-standing hyperlipidemia significantly decreased ICAM-1 levels [19]. Palmefors H et al. (2005) conducted a systematic review of the impact of physical activity or exercise on important atherosclerosis biomarkers and found strong evidence that VCAM-1 and ICAM-1 [6] decreased by aerobic exercise. No conclusive findings were discovered by the same authors concerning the impacts of physical activity on the levels of E-selectin and P-selectin. In a research by Olson TP et al. (2007), 12 months of resistance training with moderate intensity did not significantly change E-Selectin levels in healthy, overweight females [14]. The reason for the contradiction between the present research results and the results of some other researchers may be related to variations in exercise protocols, training periods, gender, age, blood pressure, CRP, subject cytokine levels, and type of supplement used in those researches. High blood pressure is one of the mechanisms to increase levels of E-Selectin. Leukocytes cannot roll on endothelial cells at low shear pressure; therefore, they cannot penetrate the space between the endothelial cells. However, with increasing shear velocity, ligand-selectin selective bindings are enhanced; this mechanism enables leukocytes to flow through the endothe-

lium at a reduced rate than the mean blood flow, thus giving the appropriate conditions for leukocyte penetration into the sub-endothelium [31]. Also, CRP levels are an efficient mechanism to increase E-Selectin levels. Because E-Selectin is not stored in granules as opposed to P-Selectin, its expression may be boosted in reaction to rise in CRP level [3]. Our research findings also showed that compared to pre-test, the mean weight and body mass index of RE groups were lowered in post-test. These changes were more apparent in CR groups than in AP groups.

Conclusion

The current research showed that both exercises of CR and AP resistance exercise trainings plus Omega3-6-9 supplementation have an efficient impact in decreasing E-Selectin but not ICAM-1 levels. However, the levels of these adhesion molecules could not be reduced by either RE or Omega3-6-9 supplementation alone. Based on the results of the present study, performing RE trainings along with intake Omega3-6-9 supplementation, make appropriate changes in the levels of E-Selectin adhesion molecule in young overweight males.

Ethical Considerations

Compliance with ethical guidelines

This article has been approved by the research project with code 2473616, dated 20/8/2016 and has been registered and ethically approved in Irandoc system with tracking number 1361610

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Authors' contributions

Study design: Keyvan Ahmadi Dehrashid, Marefat Siahkohian, Salahadin Ahmadi, Lotfali Bolboli; Data collection and analysis: Keyvan Ahmadi Dehrashid, Marefat Siahkohian, Lotfali Bolboli; Manuscript preparation: Keyvan Ahmadi Dehrashid, Marefat Siahkohian, Salahadin Ahmadi

Conflict of interest

The authors declared no conflict of interest.

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