# Research Paper: The Effectiveness of Aerobic Training With Supplementation With Vitamins C and E on Some Inflammatory and Cardiovascular Risk Factors in Overweight Men

**Bahram Abedi[1], Ali Reza Elmieh[2*], Seyed Benyamin Emam[3], Hooman Khanbabakhani[2]**

*1. Department of Physical Education, Faculty of Humanities, Mahallat Branch, Islamic Azad University, Mahallat, Iran.*
*2. Department of Physical Education, Faculty of Humanities, Rasht Branch, Islamic Azad University. Rasht, Iran.*
*3. Department of Physical Education, Faculty of Humanities, North Tehran Branch, Islamic Azad University, Tehran, Iran.*

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## ABSTRACT

**Background:** The purpose of this study was to investigate the effectiveness of aerobic training with supplementation of vitamins C and E on some inflammatory markers and cardiovascular risk factors in overweight men.

**Methods:** In this semi-experimental study, 30 overweight men were randomly assigned into three groups (n=10, AT+Sup, AT+Placebo, and control). The exercise program consisted of three sessions per week at the intensity of 60-75% of maximal heart rate for six weeks. The supplement group received 300 mg of vitamin E daily and 400 mg vitamin C tablet and the placebo group received one gelatin capsule daily during this period. Before and after six weeks of intervention, were measured interleukin-6 (IL-6), -reactive protein (CRP), Triglyceride (TG), Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), and insulin resistance index. Data were analyzed by t-test, one-way ANOVA, and Tukey post hoc test at a significance level of $P<0.05$.

**Result:** The results showed that aerobic training significantly reduced the body fat percentage, TG, and LDL in both supplement and placebo groups($P<0.05$). However, this reduction was observed in IL-6 and CRP only in the supplement group ($P<0.05$). On the other hand, the results showed that exercise and supplementation did not change the insulin resistance of subjects. The results of one-way ANOVA showed that there was a significant difference between the three groups in fat percentage, IL-6, CRP, TG, and LDL ($P<0.05$).

**Conclusion:** According to the results of this research, probably six weeks of aerobic training plus supplementation with vitamin E and C may help to reduce inflammatory symptoms, body fat percentage, and some cardiovascular risk factors.

*** Corresponding Author:**
*Ali Reza Elmieh, PhD.*
***Address:*** *Department of Physical Education, Faculty of Humanities, Rasht Branch, Islamic Azad University. Rasht, Iran.*
***Phone:*** *+98 (21) 32118971*
***E-mail:*** *elmieh@iaurasht.ac.ir*

## 1. Introduction

Obesity and overweight are related to technological advancement and changes in lifestyle [1]. Ischemic myocardial diseases are the main cause of death in various industrial and developmental societies and atherosclerosis is the most important and prevalent etiology of ischemic myocardial disease. Atherosclerosis is more acute and common in certain populations and has a severe pathology, leading to myocardial ischemia and related events even at younger ages [2]. The predictive inflammatory parameters of cardiovascular disease have been widely recognized by researchers in recent years and atherosclerosis has been recognized as an inflammatory disease. Several inflammatory markers, such as serum amyloid A, interleukin 6 (IL-6), adhesive molecules, Tumor Necrosis Factor alpha (TNF-α) and C-Reactive Protein (CRP) are effective for the prediction of cardiovascular disease. CRP is the most sensitive and strongest inflammatory indicator and predictor of the future risk of cardiovascular disease compared with other inflammatory indicators [3]. In addition to being an independent risk factor for Coronary Heart Disease (CHD), overweight and obesity indirectly increase the risk of CHD, through high blood pressure, lipid disorders, and diabetes, leading to increased levels of cholesterol and Triglyceride (TG) and reduced levels of High-Density Lipoprotein (HDL) [4].

Vitamin E has anti-inflammatory properties and eating daily vitamin E doses of 500 international units is associated with a reduction in CRP and other risk factors for cardiovascular diseases [5]. Vitamin C is beneficial in the prevention of heart problems because in addition to its properties, vitamin C is the most unstable vitamins (it is quickly eliminated by heat in the alkaline environment but stable in acidic environments) and its deficiency is quickly determined; thus, vitamin C can play a key role in the protection of LDL oxidation [6]. Physical activity leads to an increase in inflammatory cytokines, such as the Tumor Necrosis Factor-alpha (TNF-α), IL-6, and the protein of acute-phase reaction. Although some cytokines, such as IL-6 are attributed to active skeletal muscle, it has been indicated that increased body fat is exacerbating the inflammatory response to physical activity due to an increase in cytokine production. Plasma lipids in obese cases are oxidized faster than those with normal weight [7]. In obese people, lipids are targeted by free radicals due to the increased accumulation of fat in the tissues of adipose and blood. F2-isoprostanes, as eicosanoids, are obtained from arachidonic acid peroxidation. Inhibitors of the cyclooxygenase enzyme do not affect urinary F2-isoprostane levels. As a result, it is used as a bioassay in clinical studies to measure the levels of lipid peroxidation [6]. Levels of antioxidants in the body (beta-carotene and vitamins C and E circulating in the blood) are less than normal in obese people. Vitamins C and E are the best-known antioxidants that play a major role in cell function, the aging process involving vascular injury, inflammation, and neurological disorders. The inadequacy of the body's defense mechanisms for dealing with reactive oxygen species indicates that nutritional antioxidants are needed to help increase antioxidant levels and prevent cell damage in obese people.

The use of antioxidant supplements may delay oxidative stress caused by exercise. Supplementation with vitamin C decreases muscle soreness and keratin kinase and prevents oxidation of blood glutathione. On the other hand, supplementation with vitamins C and E showed a substantial increase in creatine kinase and myoglobin in the supplemented and placebo groups. However, the exercise level, its duration, and type of antioxidant were effective in obtained results. The rate of cell degradation by free radicals is affected by many different factors, such as the intensity of exercise, diet, and type of exercise [8]. During metabolism, oxidative stress leads to increased free radicals that damage the macromolecules and weaken the immune system. In individuals with normal weight, increased free radicals are exposed to the antioxidant defense system, while in obese individuals, the system is affected by multiple sources of free radicals, such as body fat. Oxidative stress in overweight men and women rises after physical activity compared with those with normal weight [9]. According to different studies and the conflicting findings, the aim of this study was to evaluate the supplementation of vitamins E and C and aerobic training on the level of cardiovascular and inflammatory risk factors in overweight men.

## 2. Methods

The present research was a semi-experimental study, in which 30 overweight men with a Mean±SD age of 33.4±4.1 years and Body Mass Index (BMI) of 26.1±71.29 $kg.m^{-2}$ were selected from 176 overweight men from a random sample. The subjects were randomly assigned into three groups based on the data from the completed questionnaires: Aerobic training+supplement group (experimental group I), aerobic training+placebo group (experimental group II), and control group [each group included 10 people]. The first and second experimental groups performed six weeks [10] of aerobic training (30 minutes in the first week and 55 minutes in the last week) at 60%-75% Heart Rate Reserve (HRR). Aerobic

training included moderate –volume and intensity aerobic interval exercise for the first and second groups, including 15 minutes of warming with various running, tensile, and tightening movements, followed by continuous running on a constant track [11]. During this period, the control group did not have a training program. The first experimental group used a pill of vitamin E (300 mg) and one tablet of vitamin C (400 mg). The second experimental group used oral pills but did not receive vitamins [12] and the control group did not receive any supplementation. Blood sampling was performed 48 hours before and after the exercise program after 12 hours of fasting in the same laboratory conditions. The average temperature of the test site was recorded at 23 to 25°C in both steps. All blood samples were taken between 8 and 9 am.

In this study, TG, LDL, and HDL were measured using an enzymatic method and using Pars Testing Company's kits (Iran) with a precision of 0.2 mg.dL-1 and an internal coefficient of variation of 2.38%. IL-6 was measured by the Enzyme-Linked Immunosorbent Assay (ELISA) method using IL-6 kits (BioVendor; Germany) with 0.92 pg.ml-1 sensitivity and intra-control and extraversion coefficients of 3.4 and 2.5%, respectively. CRP measurements were performed using an immune luminescence method using the Roche Integra Analyzer. Blood glucose was measured by blood glucose oxidase and enzymatic photometric methods using auto-analyzer (RA-1000, USA) and God-pap solution. The insulin concentration was determined using the ELISA method and direct sandwich method (Germany kit, DRG instrument Gmbh). The homeostasis model assessment (HOMA-IR) was used to evaluate insulin resistance [13]. Body fat percentage was measured by the Jackson and Pollock formula using caliper for measurement of three points of the abdomen,chest and thigh.lean body mass measured . lean body mass was calculated through body weight and body fat mass.

In this study, descriptive statistics, Kolmogorov-Smirnov test to check the normal distribution data, paired t-test for comparing pre-test and post-test data, One-way Analysis of Variance (ANOVA) for measuring the mean values of the three groups, and Tukey's test to assess the difference between the groups were used. The data were analyzed by SPSS V. 19 at the level of α=0.05.

## 3. Results

The findings of this study showed that there was a significant difference between the pre-test and post-test in IL-6 (Figure 1), fat percentage, TG, and LDL in the first experimental group (aerobic training+supplementation) and the second experimental group (aerobic training+placebo) (Table 1) (P<0.05). Regarding CRP, this difference was

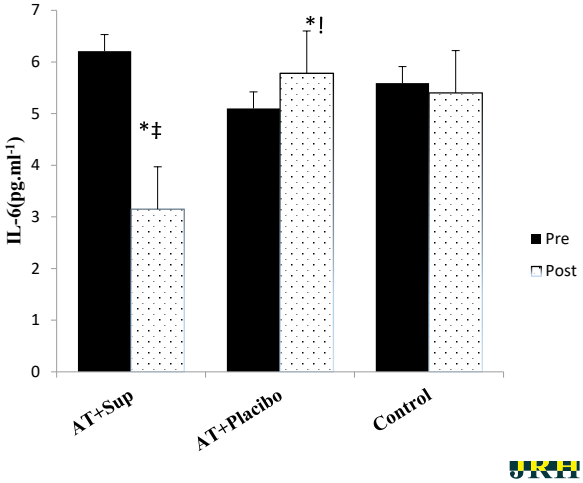


**Figure 1.** Comparison of Interleukin-6 changes before and after 6 weeks of the interventions

* Significant changes in the variables (paired t-test);

† Significant changes in the studied variables in the post-test (one-way analysis of variance);

‡ Significant changes compared with the control group (Tukey Post-Hoc test);

! Significant changes in the supplement group compared with the placebo group (Tukey Post-Hoc test).

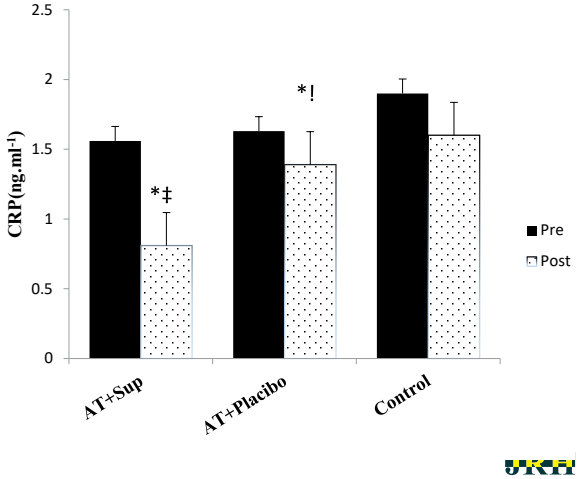


**Figure 2.** Comparison of C-reactive protein changes before and after 6 weeks of interventions

* Significant changes in the studied variables (paired t-test);

† Significant changes in the studied variables in the post-test (one-way analysis of variance);

‡ Significant changes compared with the control group (Tukey Post-Hoc test);

! Significant changes in the supplement group compared with the placebo group (Tukey Post-Hoc test).

**Table 1.** The Mean±SD of the variables of the study groups before and 6 weeks after the intervention

| Variables | Groups | Mean±SD | | P | P |
|---|---|---|---|---|---|
| | | Pre-test | Post-test | | |
| BMI (kg.m$^{-2}$) | AT+Sup | 26.5±1.3 | 24.9±3.2 | 0.079 | 0.11 |
| | AT+Placebo | 26.6±1.3 | 26.1±2.6 | 0.084 | |
| | Control | 27.01±1.3 | 27.3±3.4 | 0.091 | |
| Body fat (%) | AT+Sup | ±20.6±3.7 | 18.3±3.6‡ | 0.001* | 0.025† |
| | AT+Placebo | 21.8±2.7 | 20.2±2.5‡ | 0.001* | |
| | Control | 19.4±3.3 | 19.8±3.2 | 0.055 | |
| TG (mg.dl$^{-1}$) | AT+Sup | 190.3±88 | 160.9±76.6‡ | 0.000* | 0.001† |
| | AT+Placebo | 237.4±46 | 174±55.5‡ | 0.014* | |
| | Control | 163.6±52 | 176.7±64 | 0.112 | |
| LDL (mg.dl$^{-1}$) | AT+Sup | 114.9±23 | 109.9±22.4‡ | 0.001* | 0.025† |
| | AT+Placebo | 111.6±27 | 110.8±24.9‡ | 0.001* | |
| | Control | 107.4±23 | 111±23.2 | 0.055 | |
| HDL (mg.dl$^{-1}$) | AT+Sup | 44.7±9.2 | 843.3±8.7 | 0.055 | 0.089 |
| | AT+Placebo | 44.1±77 | 46.1±12.8 | 0.111 | |
| | Control | 39.4±7.8 | 38±9.9 | 0.068 | |
| HOMA-IR. Index | AT+Sup | 2.06±0.7 | 1.95±0.32 | 0.089 | 0.081 |
| | AT+Placebo | 1.9±0.43 | 1.9±0.39 | 0.056 | |
| | Control | 2.12±0.58 | 2.8±0.62 | 0.091 | |

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Aerobic Training and Supplement: AT+Sup; Aerobic Training and Placebo: AT+Placebo; Homeostatic Model Assessment of Insulin Resistance Index: HOMA-IR Index.

* Significant changes in the studied variables (paired t-test); † Significant changes in the post-test (one-way analysis of variance); ‡ Significant changes compared with the control group (Tukey post-hoc test); ǂSignificant changes in the supplement group compared with the placebo group (Tukey Post-Hoc test).

observed only in the first experimental group ($p<0.05$) (Figure 2). In this regard, significant difference was observed between the control group and AT+ SUP group ($P<0.05$). Other factors measured (Lean Body Mass (LBM), WHR, BMI, HDL, glucose, and insulin resistance index) were at the moderate level and showed no statistically significant difference in the pre-test compared with post-test (Table 1). The results of one-way ANOVA showed significant changes between the three groups in terms of IL-6 and CPR (Figsure 1 and 2) levels, fat percentage, and TG and LDL levels (Table 1). However, regarding glucose, insulin resistance index, BMI, WHR, and LBM although there was a difference between the mean values, it was not statistically significant (Table 1). Tukey's test results showed that the fat percentage and LDL and TG levels were significantly different between the supplement and placebo groups than the control group ($P<0.05$), while in terms of IL-6and CPR, a difference was observed between the supplement and placebo groups and the placebo and the control groups ($P<0.05$).

## 4. Discussion

The present study showed that six weeks of aerobic training and aerobic training combined with supplementation of vitamins E and C caused a significant decrease in body

fat percentage, which it was lower in the supplementation group than in the training group. The results of this study were consistent with the results of Thomson et al. who showed that aerobic exercise was effective in the body composition [14], and Jorge et al. who showed that two different types of aerobic exercises were effective in the lipid profiles of student officers in Brazil [11]. On the other hand, the results of this study were not consistent with the results of Wong et al. who showed that 8 weeks of aerobic training did not affect the body composition of subjects [15], and Sanal et al. who showed that aerobic exercise does not affect the body composition of obese and overweight boys [16]. Factors, such as gender, age, and duration of exercises can affect the outcomes of the studies.

In the present study, aerobic training and aerobic training plus supplementation with vitamins E and C caused significant changes in the levels of TG and LDL, which were higher in the supplementation group. The results of this study were consistent with the results of a study conducted by Azerbaijani et al. who showed that aerobic, resistance, and combination exercises improve strength, body composition, and lipid profiles [17], and also a study by LeMura et al. in which exercise induced changes in blood lipid parameters, such as cholesterol, TG, LDL, HDL, and very-low-density lipoprotein [18]. Abedi et al. also showed that resistance exercises and consumption of green tea caused a significant reduction in total cholesterol, TG, LDL / HDL, and very-low-density lipoprotein [3]. Interventions that increase HDL can reduce the risk of CHD by 30%-40%. The association between TG and cardiovascular disease is debatable, but there is evidence of a relationship between increased TG levels and increased risk of atherosclerotic events. TG levels have been studied predominantly in fasting state in most studies, but high TG after eating plays an important role in atherosclerosis [19]. Increased levels of LDL and TG reduced the levels of HDL, which are associated with an increased risk of coronary artery disease [20].

Studies have shown that walking is associated with reduced concentrations of total cholesterol and LDL Cholesterol (LDL-C) [21]. Short time endurance training with moderate intensity causes change in significant increase in Lecithin-Cholestrol Acyl Transferase (LCAT) enzyme activity, HDL serum concentration.

This enzyme is responsible for the transfer of CETP to other lipoproteins. These changes may be related to other mechanisms, such as changes in the concentration of plasma hormones, lipoprotein lipase, and other factors [22]. The decrease in LDL after aerobic training can be due to increased activity of the lipoprotein lipase enzyme, which increases the catabolism of the lipoproteins [23]. It has also been shown that during endocrine aerobic training, there is an increase in epinephrine, norepinephrine, growth hormone, and cortisol, and increased lipid oxidation and increased recall and use of free fatty acids provide the energy needed, leading to a reduction in body fat [11].

The findings of this study showed that supplementation with vitamins E and C plus aerobic training had a significant effect on IL-6 and CRP in overweight men. Yousefipoor et al. stated that 8 weeks of aerobic training and concurrent (aerobic-resistance) exercise significantly reduced insulin resistance index and fasting blood glucose in both groups but serum IL-6, weight, and BMI were not significantly different in the groups [24]. Studies have shown that long-term exercise can reduce CRP directly by reducing the production of cytokines in fat, muscle, mononuclear cells, and indirectly by increasing the tolerance of insulin, which all enhance endothelial function and weight loss. Studies on the effects of different levels of activity show that although exercise can cause short-term vascular inflammation complications, long-term exercise acts as an anti-inflammatory agent [13].

## 5. Conclusion

Based on the findings of this study, 6 weeks of aerobic training plus vitamins E and C supplementation can significantly reduce the fat percentage, TG, LDL, IL-6, and CPR. Therefore, aerobic exercise, along with taking vitamins E and C supplements, possibly affect the reduction of inflammatory markers and some cardiovascular risk factors. It can be concluded that supplementation of vitamins C and E along with aerobic training can be effective in coping with inflammatory responses (through IL-6 and CPR).

## Ethical Considerations

### Compliance with ethical guidelines

The study was approved by the Ethics Committee of Islamic Azad University, Rasht Branch (IR.ISU.RASHT.REC.1395.36).

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## Authors' contributions

Study design: Ali Reza Elmieh, Seyed Benyamin Emam; Data collection and data analysis: All authors; Manuscript preparation: Ali Reza Elmieh, Bahram Abedi.

## Conflict of interest

The authors declared no conflict of interest.

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