Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2012, 3 (2):944-950
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

The Influence of Vitamin E and C Supplementation and Strenuous aerobic training exercise on Plasma Cytokines in College male Students

Torksameni Arash.[1] Nikbakht Hojjatollah.[1] Ebrahim Khosro.[2] Dobkhti Faramarz.[3]Ateieh Aarabi.[4]

[1] Department of Physical Education and Sport Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Shahid Beheshti University Tehran, Iran
[3]School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran
[4]Department of Physical Education and Sport Science, Zanjan Branch, Islamic Azad University, Zanjan, Iran

---

ABSTRACT

The purpose of this study was to examine the influence of vitamin E and C supplementation and strenuous aerobic training exercise on plasma cytokines in college male students. Twenty two non-active college male students (age: 24.86 ± 4.96 year, height: 173.14 ± 4.96cm, weight :68.18 ± 8.58kg and VO_2max: 48.90 ± 5.91 $ml^{-1}kg^{-1}min$) were randomly assigned to either an antioxidant supplementation(AS) or placebo(PLA) group. The subjects received 400 IU Vitamin E and 1000 mg Vitamin C (AS group) or 1400 mg cellulose combination (PLA group) with dinner per day in a two-blind design and performed a strenuous aerobic exercise training until exhaustion with %75 of VO_2max, 5 days per week for a period of 4 weeks. Blood was collected pre and post exercise .The tests of aerobic power (Bruce test), body composition (skin fold) and blood TNF-α & IL-6 were measured by Elisa method before and after the intervention. Antioxidant Supplementation significantly decreased TNF-α & IL-6 in AS group compared to the PLA group (P≤0.05). It was concluded that in untrained humans vitamin E and C supplementations and strenuous aerobic training exercise decrease plasma cytokines.

Key Words: cytokines, Antioxidant, Vitamin E and C and Strenuous Aerobic Training Exercise.

INTRODUCTION

Oxygen as a general electron acceptor is allowed to use the energy from aerobic organisms in food, such as carbohydrates, fats and proteins. It has experimentally been shown that this catabolic process can produce samples of oxygen free radicals and other reactive oxygen. During exercise, cellular oxygen consumption and whole body oxygen consumption is increased to 20 times higher than the rest in the muscles [27]. Increased oxygen consumption, increases electron transport in the electron transport chain and thereby increases the production of free radicals. 2 to 5% of the total oxygen consumed is converted to free radicals [21]. This may lead to oxidation of vital compounds of the cells and cell membranes [8] Naturally low levels of reactive oxygen and

nitrogen species (RONS) production is essential for maintaining body function [32], so it is important for consistency of innate immune system to produce body anti-oxidative defense [23,34]. Excessive RONS production can lead to oxidation of lipids, proteins and nucleic acids inside the cell and potentially alter the normal function of cells [18]. It activates natural antioxidant defense in the body. It seems that the body is deficient in antioxidant during severe and prolonged exercise, perhaps excess free radicals due to the lack or absence of antioxidant or higher free radical production. These free radicals result in increases levels of cytokines including TNF-α and IL-6 [30]. Activities that will lead to changes in the cytokine include strenuous exercise and long-distance running [7] treadmill running [11] or cycle ergometry [28] and resistance exercise, such as wrestling increase the level of circulating tumor necrosis factor (TNF-α),and interleukin [IL-6].

Cytokine secretion Capacity decreases in athletes when compared to the group of non-athletes in response to stimuli [16], it has been shown that ROS are stimuli for exercise-induced cytokine production in non-athletes, and monocytes have no role in this process [30]. Most studies attempting to show a reduction of inflammatory marker by different methods such as the effect of exercise [24], including a 12 month training period [3], and 28 days training that doesn't decrease IL-6[2]. On the other hand, it is well accepted that the health benefits of exercise are enhanced by positive dietary modification [33]. All the damaged or dead cells caused by oxygen free radicals, activate the immune system where antioxidant such as hydrophobic vitamin E and hydrophilic vitamin C stop or minimizes this reactive process by restoring barrier [15].

In support of this evidence, some researchers investigated the anti-oxidative effect on inflammatory factors. Fisher (2004) showed that vitamin E and C, decrease IL-6 response to exercise by preventing the release of IL-6 from contracting skeletal muscle [9].Vincent (2004) observed that the anti-oxidation and oxidative stress induced by exercise in overweight people as well as markers of inflammation and lipid pr-oxidation may often be reduced with antioxidant. The other studies in this area have not been able to show the relationship between anti-oxidant and a significant reduction in inflammatory markers. Mastaloudis (2004) concluded that consumption of high anti-oxidation of exercise helps preventing oxidation but does not affect on inflammatory markers [1]. Peterson (2001) found that the use of anti-oxidant has no advantage compared with the placebo group [13]. Teixeira (2009) examined the effect of the supplements with exercise in a 28-day period and showed that supplementation whit exercise has not reduced IL-6[31].

Regarding this point that in the previous studies the different kinds of supplements haven’t been used and the consumption of anti-oxidant and done activities are different; therefore, it seems that the expanding investigations should be done to cover all aspects of this field of study. Nowadays, using of supplements in sport is more important and in long and strenuous exercises researchers attempt to improve the application of athletes by using the supplements along with the activity. So far the most studies have evaluated the effects of a single strenuous aerobic training exercise on pro- inflammatory Cytokines, but in the recent study attempts to investigate a period of strenuous aerobic training exercise with supplements on the above-mentioned pro-inflammatory factors. So the main question is whether the use of combination of vitamin E and C with several strenuous aerobic training exercise is effective on production of plasma TNF-α and IL-6 in non-active people?

MATERIALS AND METHODS

Study design

This was a randomized, double-blind, controlled study, with pretest and posttest design carried out with PLA and AS.

Figure 1. Research Project

Subjects

Twenty two non-active College male Students volunteered(age: 24.86 ± 4.96 year, height: 173.14 ± 4.96cm, weight: 68.18 ± 8.58kg and VO_2max: 48.90 ± 5.91 $ml^{-1}kg^{-1}min$) were randomly assigned to either an AS (antioxidants with exercise 12 subjects) or PLA (placebo and exercise 10 subjects) group that were selected from healthy subjects meet the requirement (non-athlete, not heavy and continuous physical activity in the past six months, no use of vitamins and supplements, maintaining physical health, and all subjects refrained from exercising or any other strenuous activity for 24 h before testing). Testing was always performed at the same time of the day (to avoid circadian rhythm variation effects in the cytokine levels) after a carbohydrate-rich breakfast, which had approval from the Azad zanjan University Ethical Advisory Committee. All subjects were informed verbally and in writing about the nature and demands of the study, and subsequently completed a health history questionnaire and informed consent .None of the subjects participated in regular exercise training or competitive sports activities or had febrile illness during the month before testing.

Preliminary Testing

All participants performed preliminary treadmill- based test at least 10 days prior to the main trial and two days after the blood sampling. Briefly, a Bruce Test to determine VO_2max and also an incremental maximal running test to determine the relationship between running speed and oxygen uptake were taken and also two main trials on separate occasions separated by 4 week.

Each subject performed two exercise sessions before and after the research. In the morning of the trial, subjects go to the laboratory at 9 AM. After subjects rested for 10 min, an indwelling catheter was inserted in a forearm vein, and a resting (baseline) venous blood sample was taken. Subjects exercised with%75 their VO2 max until exhaustion preceded by a 10-min warm-up period. Blood samples were collected at the end of the exercise sessions. Two day after completing the 4week training and supplementation, they were subjected to an exercise session of the same method (with%75 their VO2 max until exhaustion after a 10-min warm-up period).

Exercise Protocol

The subjects were training with%75 VO2 max until exhaustion in each session which done in the afternoon, 5- times in the week for 4-weeks. Due to the long running time, and subjects was non-active, every 20-minutes they could walk 5 minutes. Warm up at the beginning and cool-down at the end of exercise took place.

Supplementation

Supplementation was two capsules daily (each capsules=700 mg) taking with their diner. Supplements were prepared by the school of Pharmacy, Zanjan university of Medical Science. The SA tablets contained 400 international units (IU) of vitamin E and 1000 mg vitamin C and the 1400 mg capsules contained derivatives of cellulose in a double blind trial for 28 days. The supplements were taken in the 4-week training. The reason use of this combination of vitamin C content in the present study is consistent with research that found positive results [1, 12, 13] and vitamin E content showed the best levels of vitamin E creates in the blood [14] and the amount of research that have a positive effect [5, 9].

Dietary Analysis

To determine whether nutritional intake was different between groups or over time, 3-day dietary record forms were provided for each participant with standard instructions on how to complete the record before each blood sampling. Participants were instructed to estimate of foods using household measurements (volume) as described in national dietary guidance by the dietitian to show how to fill notes, whether or not alter their usual eating using the software FOOD WORK between macronutrients and micronutrients were assessed in two groups (Table 2).

Blood Sampling

Blood samples were collected from participants' venous catheter into 5cc EDTAK3 Vacutainer tubes before and immediately after strenuous aerobic exercise test. Plasma samples were immediately frozen and stored at -80 °C until analysis. Blood samples were analyzed for plasma levels of TNF-α and IL-6, measured with commercially available high-sensitivity ELISA kits. All assays were performed in duplicate. The intra-assay coefficient of variation was %10 for the two cytokines tested. Samples from the same subject obtained before and after 4-week exercise and supplementation were always analyzed in the same assay.

Statistical Analysis

Results are expressed as means ± Standard error, and $p<0.05$ was considered to be statistically significant. A dependent t test was used to compare results within the groups and an independent T test was used to compare results between groups.

Table 1. Subject characteristics at baseline after 4-week strenuous exercise with antioxidant supplementation. Values are presented as means ± standard error

	SA Group		PLA Group	
	First	Second	First	Second
Age (years)	25.1±4.28	25.1± 4.28	24.5±5.89	24.5± 5.89
Body weight (kg)	67.33±8.23	66.5± 8.48	69.2±9.30	68.38±9.72
Height (cm)	173.83±6.16	173.83±6.16	172±3.56	172± 3.56
BMI (kg /m^2)	22.33± 2.80	22.09± 2.98	23.1± 3.66	22.88± 3.98
Fat (percent)	12.64 ± 6.46	11.05± 6.04	12.02± 6.69	10.28± 6.23
VO_2 max (ml / kg / min)	48.33 ± 6.33	58.27± 5.36	49.6 ± 5.62	55.77± 6.03

AS, Antioxidant supplementation ; PLA, Placebo

RESULTS

Subjects characteristics (age, weight, height, BMI, percent body fat and VO_2 max) before and after testing using t-test had not significant differences between the two groups (Table 1). As is shown in Table 2 the calories, carbohydrates, proteins, fats, vitamins C and E intake in the

experimental and PLA before and after the test was evaluated using t-test showed significant differences.

Table 2.Average dietary intakes of SA Groups and PLA Groups 3days before two blood sampling. Values are presented as means ± standard error

	SA Group		PLA Group	
	First.B	Second.B	First.B	Second.B
Energy intake (kcal)	2664 ± 85	2616 ± 67	2684 ± 68	2677 ± 59
Carbohydrates (g)	332 ± 16	339 ± 16	337 ± 12	331 ± 13
Protein (g)	126± 12	124 ± 11	129 ± 13	132 ± 13
Fat (g)	91 ± 7	84 ± 11	92 ± 7	89 ± 8
Vitamin C (mg)	21 ± 112	20 ± 119	23 ± 125	25 ± 127
Vitamin E (mg)	9.6 ± 2.6	11.18± 3.09	10.66± 3.31	9.77 ± 2.53

AS, Antioxidant supplementation ; PLA, Placebo
First.B, First Blood Sampling; Second.B, Second Blood Sampling

Table 3. Pre and post exercise Inflammatory cytokine measures in SA Group and PLA Group at baseline, after 4-week strenuous exercise with antioxidant supplementation. Values are presented as means ± standard error

	SA Group		PLA Group	
	B	A	B	A
IL-6 (pg/mL) pre	1.57±0.26	6.48±1.48	1.55±0.22	6.31±2.11
TNF-α (pg/mL) pre	1.48±0.19	2.80±0.23	1.47±0.18	2.70±0.25
IL-6 (pg/mL) post	1.52±0.29	5.05±1.45*	1.60±0.17	6.42±1.25
TNF-α (pg/mL) post	1.60±0.23	2.48±0.25*	1.44±0.16	2.78±.17

AS, Antioxidant supplementation; PLA, Placebo
B, Baseline; A, After 4-week strenuous exercise with antioxidant supplementation
Pre, Pre exercise; Post, Post exercise
**: Significant difference*

DISCUSSION

This study examined whether 4-week use of anti-oxidants with exercise training reduces level of pro-inflammatory cytokines in College male Students. Studies of Cytokine response to physical activity in recent years have shown that TNF-α & IL-6 in contracting muscle, largely increased by ROS production [6].It has also been shown that taking anti-oxidant supplements can reduce pro-inflammatory cytokine [11,30]. In this study shows that the use of anti-oxidation with strenuous aerobic training exercise leads to a significant reduction in levels of IL-6 and TNF-α (%35.47 and %16.97 respectively) after 4week.

Exercise is associated with increase of ROS levels inside and outside the muscle cells and vascular components that are involved in activities. ROS as mediators of signal transduction pathways [10] are able to produce cytokines from different types of cells [19]. Result of this study shows that taking oral anti-oxidant with significantly reduced exercise-induced cytokine response to strenuous aerobic training exercise along with the role of ROS are a mediator for the production of cytokines. This indicates that ROS as important mediators of signal transduction pathways for cytokine induced exercise response. It should be noted that cytokines are rapidly up regulated in two levels of translational and post translational levels [30] and the kinetics of ROS production for signaling can be used in such a quick response.

Fisher et al (2004) used combination of 500 mg vitamin C and 400 IU of alpha tocopherol on IL-6 release from skeletal muscle in young men, and concluded that the supplements prevented the release of IL-6 muscles due to inhibition of plasma F2-isoprostanes (marker of lipid peroxidation) [9]. It has also been shown in animal model that treadmill exercise, increased level

of TNF-α in heart muscle in the presence of anti-oxidant, while no increase in cytokine production due to the elimination of ROS observed [25]. Anti-oxidation reduction in response to cytokines, observed in different types of conditions such as burn trauma, hemorrhagic shock, inflammatory bowel disease. Overall, it appears that the production of cytokines which mediated by oxidative stress is a general process in health and disease [30].

Cytokine transcription factor NF-kB activity, usually through mediation of the ROS can be produced, although other mechanisms may activate the other transcription factors and loss of membrane-bound TNF-α molecules formed by the ROS mediated [32]. It shows supplementation of anti-oxidant, NF-kB activity in heart muscle cells that has been affected by oxidants. The cytokines are usually induced through ROS- mediated activation of the transcription factor NF-B, although other mechanisms might be operating as well, such as activation of other transcription factors and ROS-mediated shedding of preformed membrane bound TNF-α molecule [30]. It was found in vitro results of ROS-dependent IL-6 production from myocytes through activation of NF-kB [19] are in accordance with this proposed mechanism, because muscles are the major IL-6-producing tissues during exercise.

Few studies have examined the effects of supplementation with exercise on cytokine caused by strenuous exercise, and some studies have failed to show positive effects of these factors on cytokine [13, 14, 16, 26]. The subjects of this study were athletes with regular physical activity which led to promote the immune system and anti-oxidative enzymes [17, 22], so it effect of supplemental anti-oxidative on inflammatory agents. Studies that have achieved positive results [4, 20] this study also showed that supplementation of anti-oxidative effects of strenuous aerobic exercise can be associated with a reduction in the cytokine of non-active people.

In this study, we used vitamins scavenger of ROS. Vitamin E is a fat soluble anti-oxidant in the cell membrane and is effective in the special quenching free radicals produced in biological membranes of mitochondria. This vitamin inhibits lipid peroxidation by reaction with different radicals of oxygen -containing, singlet oxygen, products of lipid peroxidation and super oxide radicals to form of relatively harmless tocopherol radical.Vitamin C is a water-soluble anti-oxidant, in cytosol and extracellular fluids that can be directly clear superoxide, hydroxyl radicals and singlet oxygen and participate mutually with tocopherol radical to re-production of reduced tocopherol [22, 29].

Because of various sources of ROS is in the cells, like the electron transport chain, cytosol NADH oxidase and xantin oxidase [22], there seems that the combination of detergents and inhibitors of ROS production is more effective than any single. In fact, defense against oxidative stress is related coordinate between the anti-oxidation [10]. In this study, we used combination of anti-oxidation, which leads to a reduction in ROS induced by exercise in humans. Although not measured oxidant stress in subjects, probably a combination of anti-oxidation in response to exercise was effective in reducing oxidative stress. Reduced cytokines in response to exercise is probably due to decreased production of ROS.

The results showed that anti-oxidant supplementation can reduce pro-inflammatory agents related to strenuous aerobic training in non-active subjects. Given that the anti-oxidative system is sometimes unable to copes alone oxidative stress with strenuous aerobic exercise and the body need for the supplement Vitamin does not supply, it appears that the use of supplements for people who participate in Long-term strenuous exercise and, especially non-active people is essential. According to the results supplementation for this group of people is recommended. In this regard, more research should be done on all factors influencing changes in the immune

system, including measurement of hormone changes, oxidative stress indicators, and types of training on the system with anti-oxidant supplement.

REFERENCES

[1] A Mastaloudis, J Morrow, et al. *Free Radic Biol Med*, **2004**, 15, 1329 – 41
[2] A Oberbach, A Tonjes, et al. *European Journal of Endocrinology*, **2006**, 154, 577–585.
[3] A Oberbach, S Lehmann, et al. *European Journal of Endocrinology*, **2008**,159, 129–136.
[4] A P Brendan, R Callister, et al. *British Journal of Nutrition*, **2009**, 1-6.
[5] A Singh, D Papanicolaou, L Lawrence, et al. *Med Sci Sports Exerc*, **1999**, 31, 536-542.
[6] A Zembron-Lacny, M Slowinska-Lisowska, A Ziemba. *Physiol Res*, **2010**, 59, 239-245.
[7] BK Pedersen, L Hoffman-Goetz. *Physiol Rev*, **2000**, 80, 1055–1081.
[8] B Halliwell. *Med Biol*, **1984**, 62(2), 71-77.
[9] C Fischer, N Hiscock, et al. *J Physiol*, **2004**, 558, 633– 45.
[10] CK Sen, L Packer. *Am J Clin Nutr*, **2000**, 72, 653S–669S.
[11] DA Papanicolaou, JS Petrides, C Tsigos, et al. *Am J Physiol Endocrinol Metab*, **1996**, 271, E601–E605.
[12] D C Nieman. *J Appl Physiol*, **1997**, 82, 1385 – 1394.
[13] EW Petersen, K Ostrowski, T Ibfelt, et al. *Am J Physiol*, **2001**, 280, C1570– 5.
[14] G J Kee-Ching, Y Chung-Shi, et al. *Am J C/ in Nutr*, **l996**, 64, 960-5.
[15] HB Demopoulos, JP Santomier, et al. Sport, Health and Nutrition. Champaign, IL: Human Kinetics, **1984**, 139–189.
[16] H Gabriel, G Rothe, et al. *Int J Sports Med*, **1997**, 18, 136–141.
[17] H Miyazaki, S Oh-ishi, et al. *Eur J Appl Physiol*, **2001**, 84, 1–6.
[18] I Dalle-Donne, R Rossi, et al. *Clin Chem*. **2006**, 52(4), 601-623.
[19] I Kosmidou, T Vassilakopoulos, et al. *Am J Respir Cell Mol Biol*, **2002**, 26, 587–593.
[20] J G Cannon, SN Meydani, et al. *Am J Physiol*, **1991**, 260, R1235_40 .
[21] K Fisher-Wellman, RJ Bloomer. *Dyn Med*, **2009**, 8(1), 1.
[22] LL Ji. *Proc Soc Exp Biol Med*, **1999**, 222, 283 – 292.
[23] LL Ji, MC Gomez-Cabrera, J Vina. *Ann N Y Acad Sci*, **2006**, 1067, 425-435.
[24] M peeri, M Assarzadeh, et al. *Annals of Biological Research*, **2011**, 2 (5), 213-220.
[25] N Yamashita, S Hoshida, K Otsu, et al. *J Exp Med*, **1999**, 189, 1699–1706.
[26] P Babaei, FRahmani-Nia, et al. *Journal of Clinical and Diagnostic Research*, **2009**, (3)1627-1632.
[27] P O Astrand, K Rodahl. Physiological basis of exercise. New York: McGraw Hill Book Company, **1986**, 170–175.
[28] S Rhind, G Gannon, R Shephard, P Shek. *Cytokine*, **2002**, 19, 153–158.
[29] S V Kumar, G Saritha, Md Fareedullah. *Annals of Biological Research*, **2010**, 1 (3), 158-173.
[30] T Vassilakopoulos, M-H Karatza, P Katsaounnou, et al. *J Appl Physiol*, **2003**, 94, 1025–32.
[31] VH Teixeira, HF Valente, SI Casal, et al. *Med Sci Sports Exerc*, **2009**, 41(9), 1752-1760.
[32] W Droge. *Physiol Rev*, **2002**, 82, 47– 95.
[33] W J Evans. *Am J Clin Nutr*; 72(suppl), **2000**, 647S – 52S.
[34] Z. Radak, HY.Chung, E. Koltai, et al. *Ageing Res Rev*, **2008**, 7(1), 34- 42.