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Original Article

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The Effect of Vitamin C and E Supplementation on CRP, IL- 6, Lymphocyte, Cortisol and Lactate Response Following one Aerobic Training Session

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Abstract

Introduction: The aim of this research was to compare the effect of a 15-day vitamin C and E supplementation on C reactive protein (CRP), interleukin- 6 (IL-6), cortisol, lymphocyte, and lactate response to a single aerobic training session. Methods: Thirty male college students of Islamic Azad University-Fars Science and Research Branch of with mean age of 21/53±2/99 years, weight 70/03±10/52 kg and height 176±5/97 cm voluntarily contributed in this research. First aerobic power of all subjects was estimated and the data was used to divide the subjects into three 10-persons groups (vitamin C group, vitamin E group and placebo group). A matched study design was used; hence, all target variables were measured before and after the treatment after the subject ran for 30 minutes on treadmill intensity of 75. The data was analyzed using ANOVA, ANOVA repeated measure and dependent t test with bonferroni adjustment ($p \leq 0.05$). Findings: Results showed that the 15-days vitamin C and E supplementation had no significant effect on the level of CRP, IL-6, cortisol, lymphocyte, and lactate at rest, after exercise and one hour after exercise. Conclusion: Further research is required to explore the impact of vitamin C/E administration on the level of CRP, IL-6, cortisol, lymphocyte, and lactate in different exercise conditions, using higer doses of these substances.

Keywords: Exercise, Vitamin C, Vitamin E, CRP, IL-6, Lymphocyte, Lactate, Cortisol

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1. Introduction

Human body is usually affected by bacterial environment (Nieman, 1994). These microorganisms have the potential for uncontrolled proliferation, pathologic injuries and finally killing their host. However, most infections are of short life time and cause less chronic injury. This is due to the function of immune system in battle with infectious elements. Studies show that physical activity has different effects on various body systems. In most situations physical activity and exercise have positive role in support the function of these systems, but this issue is not the case for human immune system (Hoffman et al., 1994). Early studies have indicated that body fatigue has relationship with raise in diseases. In addition, it is observed that athletes during intense training and important competitions are more susceptible to special diseases (Nieman, 1994; Nieman, 1989). Prevalence of upper respiratory tract infection (URTI) may increase following long-term and high intensity trainings (Bishop et al., 2006; Peters et al., 2001). Increase in concentration of hormones due to physical stress of hypothalamus- pituitary- adrenal (HPA) axis such as cortisol, adrenalin and some cytokines may contribute to development of disorder in immune system (Davison et al., 2005; Li T-L, Gleeson, 2005; Li T-L, Gleeson et al., 2004; Morozov et al., 2003; Nieman et al., 1997; Scharhag et al., 2002). Therefore, antioxidant supplements can affect function of immune system by reduction of release of hormones due to physical stress of HPA axis and oxidative stresses due to training (Peters et al., 2001; Robson et al., 2003). The Performance of anti-oxidative defense system of endurance trained persons increase after training, however, this change may not be enough to cope with oxidative stresses due to acute and long-term exercise trainings (Peake, 2003; Steensberg et al., 2003). High dose anti oxidative supplements (1500 mg for seven days) can reduce prevalence of infection in successful marathon athletes (Peters et al., 2001; Peters et al., 1993). Nevertheless, there is no agreement on reduced effect of oxidative stresses on immunohormonal changes during and after long-term exercise trainings (Nieman et al., 1997; Nieman et al., 2002). Indeed, in some cases, antioxidative supplements can allevaiate the oxidative stresses resulted from exercise (Peake, 2003; Nieman et al., 2004). Intensive exercises may lead to severe tissue injuries in muscles and respiratory system, so that they may induce expression of various inflammatory and pro inflammatory cytokines such as interleukin- 1 (IL- 1), interleukin-2 (IL- 2), interferon gamma (INFγ), tumor necrosis factor- α (TNF-α) and C- reactive protein (CRP) (Li T-L, Gleeson, 2005; Agha alinejad, 2002). The level of protein CRP increases in response to injury, stress and disease. CRP is the most important protein of acute phase that releases from liver in response to various lesions such as surgery, tissue injury, inflammation and exercise. This expression of this protein indicates a systemic inflammation situation. Many studies have shown reverse relationship between CRP and exercises (Nieman et al., 2002). CRP is a pre-awaring factor of cardiovascular diseases in men and women. Common exercises reduce the amount of rest CRP (Aguilo et al., 2007). Nevertheless intense and long-term exercises make significant increase (more than 20 times) in CRP concentration. IL- 6 may be produced by different cells especially stimulated monocyte- macrophage and activated T and B cells. IL-6 has wide range of activity such as stimulation of growth and production of antibody. IL-6 is a major cytokine which is responsible for release of acute phase proteins from liver. Exercise may induce stimulation of IL-6 production. Hare et al. reported that increased production of this substance by monocyte activated by LPS (approximately 65 percent), two hours after exercise (one hour exercise with 75 percent of Vo2max in untrained people) (Laurel, 1999). Associated with these findings, evidence shows that

vitamins C and E can affect immune system. The present study seeks to provide insight into the interrelation of exercise and vitamin C/E consumption in regulatory response of the immune system.

2. Methodology

Subjects

A quasi experimental study was carried out in 2011. Thirty male college students of Islamic Azad University- Fars Science and Research Branch were randomly selected and asked to complete a questionnaire about their health history. Informed consent was obtained for subject participation in the study. Demographic characteristics of subjects present in Table 1.

Protocol

First a number of physiological characteristics of all subjects were measured including height, weight and aerobic power (by cooper test) of all 30 volunteer subjects. Then subjects were then divided into three groups of equal size (vitamin C group (group C), vitamin E group (group E) and placebo group (group P)) base on their aerobic power. Three days later, seven CC blood samples were taken from subjects' anticubital vein after a 15 minutes rest. After five minutes of warm up, each subject ran 30 minutes on treadmill with intensity of 75% of heart rate reserve. Immediately the exercise and one hour later again new blood samples were taken. Subjects of group C were administered one pill of vitamin C per day containing 500 mg of the substance. The group E subjects were received one pill of vitamin E per day containing 400 UI vitamin E. The group P subject received equivalent amount of placebo per day. During the 15-days supplementation the subject were asked not to exercise. To control the acute effects of regimen on dependent variables the participants were asked to use a similar regimen 24 hours before and 24 hours after the test. Carvonen formula was used to measure the exercise intensity (75 percent of heart rate reserve):

Exercise heart rate = 75 % (maximum heart rate - rest heart rate) + rest heart rate

Blood sampling

All lab tubes contained ethylenediamine tetraacetic acid (EDTA) for prevention of blood clotting. Blood samples were centrifuged 10 minutes by speed of 2000-3000 rpm for separate serum. To measure CRP, IL-6 and cortisol sandwich ELISA method was used by Diagnostic Biochem Canada (DBC) kit, ELISA method by kit of Bender Med Systems Company and RIA method by Immuno TECH, IM1841 kit respectively.

Statistical analysis

The data was summarized using descriptive statistics. To compare the difference between CRP, IL-6, lymphocyte and cortisol one way ANOVA and the post hoc test Tukey was employed. For review the changes of CRP, IL-6, lymphocyte and cortisol within each group used repeated measure ANOVA test. In case of existence of significant difference dependent t test was used with bonferroni adjustment ($p \leq 0/05$).

Results

Results of present study showed that a 15-days administration of vitamin C had no significant effect on rest CRP ($F_{2,27}$)=1/801, p=0/18), after exercise ($F_{2,27}$)=2/256, p=0/12) and one hour after exercise ($F_{2,27}$)=2/459, p=0/10). Similar intervention, also did exert significant effect on rest IL-6 ($F_{2,27}$)=0/077, p=0/92) and one hour after exercise ($F_{2,27}$)=0/424, p=0/659). In addition, a 15-day administration of vitamin C and E did not significantly affect the rest lymphocyte ($F_{2,27}$)=0/072, p=0/931), after exercise ($F_{2,27}$)=1/336, p=0/280) and one hour after exercise ($F_{2,27}$)=0/031, p=0/970). Similarly, no significant effect on rest cortisol ($F_{2,27}$)=0/390, p=0/681), after exercise ($F_{2,27}$)=0/271, p=0/765) and one hour

after exercise ($F_{2,27}$)=1/623, p=0/216). was observed for administrating vitamins C and E. Finally, a 15-day administration of vitamin C and E did not significant alter the rest lactate ($F_{2,27}$)=0/405, p=0/671), after exercise ($F_{2,27}$)=1/679, p=0/205) and one hour after exercise ($F_{2,27}$)=0/489, p=0/619).

Table 1. Demographic characteristics of cases

Variable / Group	Age (M±SD)	Height (M±SD)	Weight (M±SD)
vitamin C	21/7±4/49	175/60±7/44	68/50±9/73
vitamin E	21/5±2/79	172/80±3/45	67/15±7/66
Placebo	21/54±0/93	179/55±43/4	72/95±13/33

Table 2. Statistical analysis of CRP, IL-6, lymphocyte, cortisol and lactate of research groups in pre and post test

Test time	Variable	Group	Rest (M±SD)	After exercise (M±SD)	One hour after exercise (M±SD)
Pre test	CRP (ng/ml)	Placebo	741/6±773/68	761/9±754/54	565±529/93
		vitamin C	410/5±241/02	440/8±285/95	298±176/60
		vitamin E	924/8±1415/22	1062/3±1790/44	737/8±1236/74
	IL-6 (pg/ml)	Placebo	0/481±0/10	0/814±0/20	0/737±0/21
		vitamin C	0/487±0/13	0/980±0/31	0/789±0/33
		vitamin E	0/470±0/12	0/870±0/19	0/634±0/10
	lymphocyte	Placebo	1948/76±395/30	3991±831/28	1981/23±517/63
		vitamin C	2002/60±535/32	4310/10±913/42	1823/70±500/24
		vitamin E	1963/80±545/44	4549/90±1432	1944/50±464/30
	Cortisol (ng/ml)	Placebo	90±46/14	143/90±52/40	172/80±6 1/64
		vitamin C	98/10±29/54	142/10±43/50	163/50±53/52
		vitamin E	108±51/51	151/30±63/13	166/60±50/60
	Lactate (mg/dl)	Placebo	16/96±5/11	107/90±18/54	27/54±6/68
		vitamin C	17/03±3/65	122±31/43	31/58±8/13
		vitamin E	15/56±2/67	123/35±25/97	32/87±9/ 10

Test time	Variable	Group	Rest (M±SD)	After exercise (M±SD)	One hour after exercise (M±SD)
Post test	CRP (ng/ml)	Placebo	1686/2±2733/19	1909±3027/16	1704/1±2640/64
		vitamin C	411/2±278/64	473/3±271/3	345±269/79
		vitamin E	808/6±1147/32	858/7±1374/75	646/6±952
	IL-6 (pg/ml)	Placebo	0/592±0/25	1/132±0/54	0/868±0/24
		vitamin C	0/634±0/19	0/985±0/24	0/792±0/49
		vitamin E	0/581±0/10	0/864±0/21	0/730±0/25
	lymphocyte	Placebo	2004/61±377/29	3887/15±573/19	2013/46±388/70
		vitamin C	1946/80±385/47	3825/70±874/88	2060/40±285/91
		vitamin E	1956/10±455/81	3784/30±806/59	2061/40±688/99
	Cortisol (ng/ml)	Placebo	75/80±43/05	113/70±53/29	131/60±53/58
		vitamin C	94/90±40/92	92/30±28/08	80/4±23/74
		vitamin E	86/40±41/66	108/10±42/16	122/50±40/40
	Lactate (mg/dl)	Placebo	15/62±4/17	103/28±17/79	25/63±8/85
		vitamin C	16/66±5/78	89/68±27/87	24/81±5/90
		vitamin E	16/57±4/71	108/08±34/45	28/96±8/03

Table 3. ANOVA test for review changes of CRP, IL-6, lymphocyte, cortisol and lactate at resting time , after exercise and one hour after exercise

variable	state		Sum of Squares	df	Mean of Squares	F	Sig
CRP	Rest	Between groups	6766364/867	2	3383182/433	1/801	0/18
		Within groups	5/073	27	1678791/856		
		Total	5/749	29			
	After exercise	Between groups	1/041	2	5204111/433	2/256	0/12
		Within groups	6/228	27	2306774/511		
		Total	7/269	29			
	One hour after exercise	Between groups	9084732/467	2	4542366/233	2/459	0/10
		Within groups	4/988	27	1847344/907		
		Total	5/896	29			
IL-6	Rest	Between groups	0/006	2	0/003	0/077	0/92
		Within groups	1/113	27	0/041		
		Total	1/120	29			
	After exercise	Between groups	0/686	2	0/343	3/743*	0/03
		Within groups	2/474	27	0/092		
		Total	3/160	29			
	One hour after exercise	Between groups	0/093	2	0/046	0/424	0/659
		Within groups	2/948	27	0/109		
		Total	3/040	29			

variable	state		Sum of Squares	df	Mean of Squares	F	Sig
Lymph	Rest	Between groups	35344/06	2	17672/03	0/072	0/931
		Within groups	6621867/80	27	245254/36		
		Total	6657211/86	29			
	After exercise	Between groups	1962887/26	2	981443/63	1/336	0/280
		Within groups	1/98	27	734766/28		
		Total	2/18	29			
	One hour after exercise	Between groups	14335/80	2	7167/90	0/031	0/970
		Within groups	6320427/40	27	234089/90		
		Total	6334763/20	29			
Cortisol	Rest	Between groups	1714/40	2	857/20	0/39	0/681
		Within groups	59351/60	27	2198/20		
		Total	61066	29			
		Between groups	1989/06	2	994/53	0/271	0/765
		Within groups	99016/80	27	3667/28		
		Total	101005/86	29			
	One hour after exercise	Between groups	10950/06	2	5475/03	1/623	0/216
		Within groups	91109/40	27	3374/42		
		Total	102059/46	29			
Lactate	Rest	Between groups	27/89	2	13/94	0/405	0/671
		Within groups	929/27	27	34/41		
		Total	957/16	29			
	After exercise	Between groups	3904/71	2	1952/35	1/679	0/205
		Within groups	31392/61	27	1162/68		
		Total	35297/33	29			
	One hour after exercise	Between groups	119/33	2	59/66	0/489	0/619
		Within groups	3296/71	27	1221/10		
		Total	3416/05	29			

* The mean difference is significant at the 0.05 level.

Table 4. Tukey test for reviewing significant differences of IL-6 between research groups after exercise

State	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
After exercise	Control	vitamin C	0/313	0/135	0/07	-0/022	0/648
		vitamin E	0/328	0/135	0/05	-0/007	0/663
	vitamin C	Control	-0/313	0/135	0/07	-0/648	0/022
		vitamin E	0/015	0/135	0/99	-0/320	0/350
	vitamin E	Control	-0/328	0/135	0/05	-0/663	0/007
		vitamin C	-0/015	0/135	0/99	-0/350	0/320

Table 5. The results of the repeated measure rest for changes of CRP, IL-6, lymphocyte, cortisol and lactate in research groups

Group	Source	Sum of Squares	df	Mean of squares	F	Sig
Placebo	CRP	1/79	1/02	1/75	2/08†	0/182
	Error	7/72	9/19	8404921/74		
vitamin C	CRP	206273/13	5	41254/25	3/69*	0/007
	Error	502496/53	45	11166/59		
vitamin E	CRP	1057921/40	1/47	715925/52	1/33†	0/286
	Error	7123399/26	13/29	535623		
Placebo	IL-6	2/58	2/03	1/27	10/92†	0/001
	Error	2/13	18/31	0/11		
vitamin C	IL-6	1/90	2/26	0/83	6/15†	0/006
	Error	2/77	20/41	0/13		
vitamin E	IL-6	1/15	5	0/23	11/67*	0/000
	Error	0/788	45	0/02		
Placebo	Lymphocyte	6/61	2/65	2/49	70/90*†	0/000
	Error	1/11	31/86	351380/06		
vitamin C	Lymphocyte	6/08	2/53	2/39	51/46*†	0/000
	Error	1/06	22/82	465889/92		
vitamin E	Lymphocyte	6/67	1/96	3/40	45/76*†	0/000
	Error	1/31	17/65	742919/45		

Group	Source	Sum of Squares	df	Mean of squares	F	Sig
Placebo	Cortisol	63768	5	12753/60	6/42*	0/000
	Error	89399/33	45	1986/65		
vitamin C	Cortisol	54304/48	2/36	22972/56	7/47*†	0/002
	Error	65382/01	21/27	3073/19		
vitamin E	Cortisol	44846/68	5	8969/33	5/57*	0/000
	Error	70131/15	45	1558/47		
Placebo	Lactate	95615/72	2/54	37574/73	155/71*†	0/000
	Error	5526/26	22/90	241/29		
vitamin C	Lactate	99303/95	2/20	45077/96	71/57*†	0/000
	Error	12486/48	19/82	629/78		
vitamin E	Lactate	116858/63	2/07	56270/11	73/19*†	0/000
	Error	14369/69	18/69	768/81		

* The men difference is significant at the 0/05 level.

†As Mauchly's Test of Sphericity was significant, Greenhouse-Geisser test used to adjust the degrees of freedom

Table 6. Pairwise comparison of CRP, IL-6, lymphocyte, cortisol and lactate in the study groups

Variable	State	(I) GROUP	(J) GROUP	Mean Difference (IJ)	Std. Error	Sig†*	95% Confidence Interval	
							Lower Bound	Upper Bound
CRP	vitamin C	One hour after exercise of post test post test	Rest of post test	112/50	27/43	0/04	4/004	220/996
IL-6		After exercise of pre test	One hour after exercise of pre test	0/353	0/056	0/002	0/132	0/574
	vitamin C	after exercise of pre test	One hour after exercise of post test	0/498	0/083	0/003	0/168	0/828

Variable	State	(I) GROUP	(J) GROUP	Mean Difference (IJ)	Std. Error	Sig†*	95% Confidence Interval	
							Lower Bound	Upper Bound
IL-6	vitamin E	after exercise of post test	One hour after exercise of post test	0/493	0/102	0/014	0/091	0/895
		after exercise of pre test	One hour after exercise of pre test	0/283	0/066	0/040	0/010	0/556
		after exercise of pre test	One hour after exercise of post test	0/394	0/077	0/014	0/077	0/712
		One hour after exercise of pre test	after exercise of post test	-0/289	0/055	0/012	-0/515	-0/062
		Rest of post test	after exercise of post test	-0/236	0/057	0/047	-0/469	-0/002
		after exercise of post test	One hour after exercise of pre test	0/400	0/075	0/010	0/092	0/708
	vitamin C	Rest of pre test	after exercise of pre test	-2307/50	171/97	0/000	-2987/51	-1627/48
		Rest of pre test	after exercise of post test	-1823/10	230/18	0/000	-2733/29	912/90
		after exercise of pre test	One hour after exercise of pre test	2486/40	262/66	0/000	1447/75	3525/04
		Rest of pre test	after exercise of post test	-1765/30	235/49	0/001	-2696/48	-834/11
		after exercise of post test	One hour after exercise of post test	1878/800	211/28	0/000	1043/32	2714/37

Variable	State	(I) GROUP	(J) GROUP	Mean Difference (IJ)	Std. Error	Sig†*	95% Confidence Interval	
							Lower Bound	Upper Bound
Lymphocyte	vitamin E	Rest of pre test	after exercise of pre test	-2586/10	372/56	0/001	-4059/32	-1112/87
		One hour after exercise of pre test	after exercise of pre test	2605/40	381/60	0/001	1096/43	4114/36
		Rest of post test	after exercise of post test	-1828/20	195/08	0/000	-2599/61	-1056/78
		after exercise of post test	One hour after exercise of post test	1722/90	202/24	0/000	923/16	2522/63
Cortisol	vitamin C	Rest of pre test	Rest of post test	-83/10	19/64	0/034	-160/96	-5/234
		after exercise of pre test	after exercise of post test	-49/80	12/53	0/049	-99/37	-0/223
		after exercise of post test	One hour after exercise of post test	44	10/56	0/037	2/21	85/78
	vitamin E	One hour after exercise of pre test	Rest of post test	-80/20	17/65	0/021	-149/99	-10/40
	vitamin C	Rest of pre test	after exercise of pre test	-64/87	8/14	0/000	-97/06	-32/67
		after exercise of pre test	One hour after exercise of pre test	73/02	7/75	0/000	42/35	103/69
		Rest of post test	after exercise of pre test	-90/42	9/32	0/000	-127/27	-52/56
		Rest of post test	One hour after exercise of pre test	14/55	2/73	0/007	3/74	25/35

Variable	State	(I) GROUP	(J) GROUP	Mean Difference (IJ)	Std. Error	Sig†*	95% Confidence Interval	
							Lower Bound	Upper Bound
Lactate	vitamin E	after exercise of post test	One hour after exercise of post test	104/97	9/98	0/000	65/50	144/43
		Rest of pre test	after exercise of post test	-79/12	9/20	0/000	-115/51	-42/72
		Rest of pre test	after exercise of pre test	12/39	3/11	0/048	0/093	24/68
		after exercise of pre test	One hour after exercise of post test	91/51	11/43	0/000	46/29	136/72
		Rest of post test	One hour after exercise of post test	-90/48	7/34	0/000	-119/53	-61/42
		Rest of pre test	One hour after exercise of pre test	17/31	2/83	0/003	6/09	28/52
		after exercise of pre test	One hour after exercise of pre test	107/79	8/22	0/000	75/27	140/30

* the men difference is significant at the 0/05 level.

†Adjusted by bonferroni adjustment

3. Discussion

Findings of present study showed that a 15-day vitamin C supplementation does not reduce rest CRP and CRP response to exercise. In is in contrast with the previous studies where a two-week vitamin C supplementation was found to reduce CRP response to exercise (Agha alinejad, 2002; Laurel, 1992). The inconsistency can be explained by the assumption that in present study selected exercise did deplete body's vitamin C storages, hence no response to vitamin C administration was observed. On the other hand, a 500 mg dose may not be adequate for reduction of CRP response where compared with the dosages

used in previous studies (Davison et al. 2006, 2007). Gleeson et al (2005), by examining the effect of vitamin C and carbohydrate supplementation before and during long-term exercises on some immunohormonal parameters (Gleeson et al., 2005) confirmed that acute vitamin C supplementation has no significant effect on rest CRP. In the study of Davison et al (Davison et al., 2007) and present study where vitamin C was administered with a 500 mg dose, no significant change in CRP level was found. By contrast, use of 1000 mg dosage in other studies has led to significant changes in CRP levels (Gladys et al., 2009). Findings of the present study showed that vitamin E supplementation could not reduce CRP at rest, after exercise and one hour after exercise. It has been stated that vitamin E may affects production of inflammation intermediators such as prostaglandins, leukotrienes and oxygen radicals that increase the release of cytokines. Previous findings show that if vitamin E supplementation is used before the exercise, it may help limit increase of inflammation intermediators and cytokines after exercise, hence, aggregation of inflammatory cells in skeletal muscles after eccentric exercises would be reduced (Laurel, 1999). Nevertheless in the present study one reasons to explain why vitamin E supplementation did not affect CRP, may be the low dosage (400 UI) compared with that in previous studies (800 UI) (Kolb, 1997; Claudio et al., 2006). Findings of present study showed that although in any groups CRP did not increase significantly after exercise, one hour after exercise CRP reduced to the level lower that during the rest. Exercises have a twofold effect: acute effect of single bout exercise in increasing CRP for several continuous days and a chronic pause in release of CRP following resumption of intense exercises. This effect is similar to the neutrophils actions. Exercises that result in increased CRP level represent effects of both the severity and duration of activity (Laurel, 1999).

It appears that the intensity of exercise prescribed in this study made less mechanical stress: the effect of this exercise (inhibition of acute phase proteins) had dominant mode and thus CRP levels have reduced. In exercises like bicycling that has more mechanical stress, acute and chronic effects are in highest level of normal range of CRP level. Nevertheless this assumption should examine more comprehensively. Differences between various studies can by due to the time between exercise stop and blood sampling. Some cytokines, in particular pre inflammatory cytokines such as IL-1, IL-6 and TNF-α release during exercise. These cytokines play important role in inflammation. Therefore, exercises may induce injury and inflammation in skeletal muscles (B Czarkowska -Paczek et al., 2005). As our results indicated, 30 minutes aerobic training with intensity of 75 percent of heart rate reserve resulted in an increase in IL-6 and IL-6 levels that did not return to initial levels one hour after exercise. This finding can be interpreted in terms of an increase in plasma concentrations of IL-6 due to sever exercises (more than 70 percent of heart rate reserve) that that engage a large group of muscles. Previous studies show that using vitamin E supplements before exercise may help limit increase of inflammatory mediators and cytokines after exercise and result in reduced accumulation of inflammatory cells in the skeletal muscle after eccentric exercise (Claudio et al., 2006). However, in our study, a 15-days vitamin C and E supplementation left no significant effect in reduction of IL-6 at rest and after exercise. This observation may be due to low doses or short period supplementation (15 days versus two months). During exercise, lymphocytes increase less than neutrophils which lead to higher neutrophils to lymphocytes ratio during and after exercise (Laurel, 1999). Studies have shown that lymphocytosis occurs during and immediately after exercise and regardless of the exercise intensity and duration (Laurel, 1999). In this study, in line with previous studies, lymphocytes significantly increased after exercise and then

returned back to resting levels one hour after exercise. Despite that, no significant differences between the groups given supplements of vitamins C and E were observed. Lymphocytosis during exercise depends on the interaction between exercise intensity and fitness level of the individual. During moderate or too short exercises, the number of lymphocytes may remain unchanged or rise to more than 50% of that during the rest. During prolonged and sub-maximal exercises, the number of lymphocytes may rise 2 to 3 times higher than the corresponding resting levels. Similar to the white blood cells, lymphocytes rise progressively along with the exercise intensity (Laurel, 1999). However, unlike the leukocytosis case, here, the duration of exercise may not be a determining indicator. Results of this study were in line with study of Shek et al (1995). These authors observed that in the first 30 minutes of running on treadmill with an intensity of 65% maximal oxygen uptake, cells doubled, then slowly dropped within 30 to 90 minutes and increased again at the end of exercise (Shek et al., 1995). We observed that vitamin C and E supplements for 15 days resulted in reduced resting and after-exercise cortisol response. However, this reduction was not significant. Studies indicated that hormones such as epinephrine and cortisol affect redistribution of white blood cells in blood flow and different parts of the body such as spleen, liver and bone marrow. Increase in cortisol secretion with regard to training capacity of individuals, depends on exercise intensity (Peters et al., 2001). Individual differences in response of glucocorticoids to exercise, especially in people who train very well, have higher influence, because cortisol is released only during hard exercises. During exercise, cortisol responds to certain level of activity and epinephrine responds to low levels of activity. Cortisol shows a lag phase after exercise then began to increase or remains at higher levels (Laurel, 1999). Increase in stress hormones of hypothalamus- pituitary axis such as cortisol, adrenaline and some cytokines may contribute to the disorders of the immune system following long-term trainings (Davison, 2005; Li T-L, Gleeson, 2005). Thus, antioxidant supplements enhance immune function of athletes by reducing the release of stress hormones of hypothalamus-pituitary axis. Results of this study were consistent with some previous findings (Nieman et al., 1997; Peake, 2003; Fischer et al., 2004), however, they contrast with some others (Davison et al., 2006; Nieman et al., 2002). Cortisol biosynthesis in the adrenal glands begins with the conversion of cholesterol to pregnenolone. This stage is the original point in the action of adrenocorticotrophin, thus, it is limited in speed and rhythm and. Since cortisol of adrenal and vitamin C is not released at the same time in response to oxidative stresses, reduction of cortisol levels with vitamin C supplements may be attributed to to other mechanisms such as the level of fitness and intensity and duration of activity before the measurement of cortisol. Based on findings of Nieman et al (Nieman et al., 1997), IL-6 is an important factor in immune responses. This factor release from contracting muscles and its level is reduces by antioxidant supplements. This cytokine stimulates increase in secretion of cortisol from the adrenal glands. Fischer et al (2004) did not find differences in gene expression of IL-6 or IL-6 in trained muscle fibers between placebo and supplement received group. It is suggested that the reduction in systemic concentrations of IL-6 is due to reduction of its release or its transport from tissue (skeletal muscles) into the bloodstream. The point of action can be plasma membrane that is required for interactions between vitamin C and E (Fischer et al., 2004). Reports show that high grade ascorbic acid in the adrenal gland inhibits steroidogenesis. In this regard it is thought that ascorbic acid by changing the structure and lipid composition of cell membranes disturbs the binding of ACTH to membrane receptors and inhibits the ACTH-dependent steroidogenesis. It is believed that ascorbic acid has key role in the production

and regulation of corticosteroid production in the adrenal gland (Agha alinejad, 2002). The incongruence between studies may be justified for several reasons such as duration, training intensity, fitness, total or free cortisol concentration, and cconsideration of an appropriate control group to measure daily changes. In comparison with total cortisol, free cortisol levels increase during intense exercise and decrease after exercise.

Our results showed that a 15-day vitamin C and E supplementation has no effect lactate accumulation in blood in response to exercise. In this regard the studies of Aguilo et al (2007) and Kimberly et al (2001) showed that Antioxidant supplements can reduce blood levels of lactic acid. However, these studies have used a combination of antioxidant supplements (Aguilo et al., 2007; Kimberly et al., 2001). Numerous studies have been conducted over the past decades about the relationship between vitamin C/E and their role in improvement of physical function. The results of these studies however remained contradictory. Studies that explored the effects of vitamin C and E supplementation on body function in animal and human groups indicated the positive effects of vitamin C and E on reduction of muscle fatigue and increase in work rate (Holden et al., 2006; Özaslan et al., 2004; Judy et al., 2006), decrease in heart rate (Judy et al., 2006), improvement of maximum oxygen consumption (Holden et al., 2006; Judy et al., 2006) and running time (Judy et al., 2006). There are also many research not finding effect for vitamin C and E supplements on the muscle endurance, tidal volume, lactic acid accumulation (Judy et al., 2006), rest heart rate (Kimberly et al., 2001; Judy et al., 2006), maximum oxygen consumption, anaerobic performance (Judy et al., 2006), aerobic capacity, speed and strength (Claudio et al., 2006). So many factors including time of supplementation, intensity and duration of exercise, exercise type and responses of each sport to target indices, dose rate, and type of antioxidant, measurement methods and test time may affect the effectiveness of vitamin C/E supplementation (Dekkers et al., 1996; Kanter et al., 1995; Powers et al., 2004).

4. Conclusion

Our results showed that a 15-days Vitamin C and E supplementation cannot alter the level of CRP, IL- 6, Lymphocyte, Cortisol and Lactate at rest, immediately after exercise and one hour after exercise with intensity of 75% of heart rate reserve. Nevertheless it has been reported that vitamin C and E may be useful in limiting the infection intensity during body stress periods. The conflicts between findings suggest the need for further studies using higher dosages to achieve a clear description of immunohormonal responses to vitamins C and E consumption.

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