EFFECTS OF VITAMIN C AND E COMBINATION ON HORMONAL, ENZYMATIC AND HEMATOLOGICAL VALUES IN BLOOD OF FORCED TRAINING BASKETBALL PLAYERS

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ABSTRACT: I investigated effects of vitamin C and E (VCE) combination supplementation on lactate dehydrogenase (LDH), creatine kinase (CK) activities, and free testosterone, cortisol and hematological values in blood of forced training of basketball players. Blood was obtained from 14 male basketball players pre-game (Group A), post-game (group B) and after 35 days on vitamin C (500 mg) and E (150 mg, VCE)/ 24 h orally) supplementation pre (Group C) and post-training (Group D). The anticoagulated blood and serum samples were obtained from all subjects. Cortisol levels were lower in forced training subjects than in control subjects, whereas there was a significant increase serum CK levels, free testosterone levels and free testosterone/cortisol ratio. Decreased cortisol values were mostly increased by exercise plus VCE supplementation although serum CK levels, free testosterone levels and free testosterone/cortisol ratio were decreased by exercise plus the VCE supplementation. There were no significant changes in white blood cells, red blood cells and platelets counts and hemoglobin and hematocrit values in the four groups. In conclusion, these data demonstrated an increase in the CK in the serum of forced training whereas there is a decrease in the free testosterone, cortisol and their ratio. However, supplementation of VCE might partially modulate for the hormonal and enzymatic stress marker profile due to strengthen the antioxidant defense system by decreasing ROS

KEY WORDS: basketball; creatine kinase; cortisol; oxidative stress; testosterone

INTRODUCTION

The human body is equipped with a complete arsenal of defences against external and internal aggressions. Those against the so-called reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide are crucial in inflammatory and antibacterial responses where they participate in physiological processes such as arachidonic acid cascade and phagocytosis [1]. The concentrations of these metabolic intermediates are actually kept under strict control by the activity of a complex defence system including enzymes and non-enzymatic species such as vitamin C (ascorbic acid), vitamin E (tocopherol) [8,17]. However, an uncontrolled production of ROS is liable to occur in several conditions leading to a situation known as "oxidative stress" where the highly ROS can attack many essential biomolecules (protein, DNA, RNA, lipids) and even cell structures, causing oxidative damages. As a matter of fact, many pathological processes are initiated or aggravated by such processes [11,16]. For several years, pharmacological investigations of exogenous compounds or therapeutical agents in exercised subjects have focused on a possible interaction with ROS (6-11) in order to assess their capacity to prevent or minimize free radical damages to biological targets. A low intake of vitamin C and E (VCE) coupled with the oxidative stress of prolonged exercise may further exacerbate the resulting oxidative damage to cellular membranes. VCE deficient diets may make athletes more susceptible to muscle damage and injury [3,10].

To support this premise, it has been demonstrated that vitamin E supplemented athletes show a decrease in CK as a measure of muscle damage after strenuous exercise [19]. Rokitzki et al. [14] supplemented male cyclist with vitamin E and found a significant reduction in CK in the serum of those supplemented with vitamin E during aerobic training, thus indicating a protective effect of vitamin E against oxidative stress- induced degeneration. In another study in which a forced training protocol was used, CK became elevated post exercise with a similar time course [19] and these results suggest a relationship between free radical production and exercise-induced muscle damage. However there are also conflict reports on the subjects. Recently, Dawson et al. [3] reported that no significant differences were found between the VCE and placebo groups for CK levels recorded after the 21 km runs. Mastaloudis et al. [10] repor-

ted that VCE supplementation appeared to have no effect on exercise-induced increases in muscle damage or recovery as measure of LDH and CK. However, whether combination of VCE supplementation to forced training basketball players confers increase of CK and LDH in basketball players is currently unknown and warrants further study.

Physical activity also influences changes in serum levels of Cortisol and testosterone, depending on the duration and the intensity of the exercise. A decrease in the testosterone/cortisol ratio has been found to be associated with the overtraining syndrome characterised by a decreased sport- specific physical performance [15]. This ratio has also now been reported to be an indicator of the actual physiological strain in training [15,18] although Harber et al. [6] reported serum testosterone, cortisol, and the testosterone/cortisol ratio did not change at any time for the circuit weight training men.

If forced training increases oxidative stress, muscle cells should be oxidized and enzymes such as CK and LDH in the blood should be increased. To test this hypothesis, basketball players were studied after forced training exercise in order to check whether exercise in the subjects increases LDH and CK and stress dependent hormones namely cortisol and testosterone and decreases hematological values. The second aim of the current study was to test the moderate dose of VCE and its possible beneficial effect on the tissue defense system by evaluating the enzymes and hormones in the blood of forced exercising subjects.

MATERIALS AND METHODS

Chemicals. All chemicals were obtained from Sigma Chemical Inc. (St. Louis, MO, USA) and all organic solvents from Merck Chemical Inc. (Darmstadt, Germany). The dietary forms of vitamin C (ascorbic acid) and vitamin E (dl-α-tocopheryl acetate) were obtained from Bayer Inc (İstanbul, Turkey). All reagents were of analytical grade.

Subjects. Fourteen male randomly selected basketball players, all members of an professional team aged 16.83±1.06 years high 188±5 cm, weighing 72.0±1.2 kg volunteered to participate in the two parts of this study after signing the specific formed consent. Anthropometric measures were taken according to Lohnman et al. [9]. In particular, standing high (cm) was measured with precision of 0.1 using a stadiometer. Body weight (kg) with light indoor clothing, without shoes, was recorded with a scale to nearest 100 g. Blood pressure was determined with an electronic instrument with which heart ratio was simultaneously measured. The training units consisted in a general warm up and stretching (about 10 min), a technical- tactical part (about 30 min), a heavy training load part including training counterattacks and simulated full or half- court basketball games (about 40 min), and finally a cool down phase (10 min) [15,19].

Experimental Design. The study was divided into two parts: In part A, the players were clinically examined and blood was drawn for laboratory tests before entering the warm up stage (group A, n=8) and at the end of forced training (group B, n=8). After the end of part A, the players were orally supplemented with vitamin E DL-α-tocopheryl acetate (150 m/24 hours) and vitamin C (ascorbic acid) 500 mg/24 hours) combination for 35 successive days [15]. Then, they were clinically re-evaluated and blood was drawn before (group C, n=6) and at the end of the training (group D, n=6) for re-determination of same antioxidant vitamin and enzymes values and lipid peroxidation levels.

Blood collection and preparation of blood samples After informed consent was obtained from all patients and control subjects, whole blood samples (total 5 ml) were taken by using a 25- gauge needle from a peripheral vein, avoiding hemolysis, into tubes in the morning hours (8:00-10:00) after an overnight fast and 30 minutes of supine rest. One ml of non-coagulated blood was used for hematological analysis. The remaining blood sample was separated into serum by centrifugation at 1500 • g for 10 min at + 4°C. All parameters were measured within 6 hours following blood taking.

Measurement of biochemical values. Serum lactate dehydrogenase (LDH) and creatine kinase (CK) enzyme activities were determined by routine kits using an autoanalyzer (Hitachi Modular Analyzer, Tokyo, Japan).

Serum hormone analysis. Cortisol levels were analyzed by Radioimmunoassay (DPC Biermann GmbH, Bad Nauheim, Germany) and the values were expressed as nmol/ml. The samples were counted in a gamma counter. Free testosterone levels were also analyzed in by using commercial kits (Free Testo RIA-CT kit, Biosource Europe S.A - Nivelles, Belgium) and its values were expressed as pmol/l.

Hematological parameter analyses.White blood cells (WBC), red blood cell (RBC) and platelet counts, hematocrit and hemoglobin values were determined using an automated blood counter (Beckman Coulter, Miami, USA).

Statistical analyses. All results are expressed as means ± SD. To determine the effect of treatment, data were analyzed using ANOVA. P-values of less than 0.05 were regarded as significant. Significant values were assessed with LSD test. Data was analyzed using the SPSS statistical program (version 9.05 software, SPSS Inc. Chicago, Illinois, USA).

RESULTS

The mean LDH and CK enzyme activities in serum in four groups were shown in Figure 1. The results showed that the CK activities in serum in group B group were significantly ($p<0.05$) higher than in group A. Supplementation of VCE to the basketball players of group D caused to decrease in the CK levels of plasma according to group B and the CK activities in plasma in the group D were significantly ($p<0.01$) lower than in the group B. There was no statistical significance in serum LDH activities in the four groups.

The mean free testosterone and cortisol levels and free testosterone/cortisol ratio (median range) in serum of four groups were shown also in Table 1. The results showed that free testosterone levels and free testosterone/cortisol ratio in serum in group B were significantly ($p<0.05$) higher than in group A although cortisol levels were incre-

TABLE 1. CORTISOL, FREE TESTESTORONE LEVELS AND FREE TESTOSTESTORONE/CORTISOL RATIO (MEDIAN RANGE) IN THE SERUM OF BASKETBALL PLAYERS BEFORE AND AFTER TRAINING AND WITH AND WITHOUT VITAMIN C AND E COMBINATION SUPPLEMENTATION (MEAN±SD)

Paramaters	Group A (pre-training) (n=8)	Group B (post-training) (n=8)	Vitamin C and E Group C (pre-training (n=6)	Supplementation Group D (post-training) (n=6)
F. testosterone (pmol/ml)	24.13 ± 4.41	31.24 ± 2.96*	20.27 ± 2.51	24.32 ± 4.49[a]
Cortisol (nmol/ml)	377.21 ± 37.31	317.61 ± 42.12*	389.34 ± 44.54	326.15 ± 44.87[a]
F. testosterone/cortisol Ratio	0.064 ± 0.011	0.100 ± 0.019*	0.053 ± 0.014	0.074 ± 0.006[a]

* - p<0.05 compared with group A,. [a] - p<0.05 as compared with group B and C.

TABLE 2. WHITE BLOOD CELLS (WBC) AND RED BLOOD CELLS (RBC) COUNTS AND HEMOGLOBIN AND HEMATOCRIT VALUES IN BLOOD OF BASKETBALL PLAYERS BEFORE AND AFTER TRAINING AND WITH AND WITHOUT VITAMIN C AND E COMBINATION SUPPLEMENTATION (MEAN±SD)

Paramaters	Group A (pre-training) (n=8)	Group B (post-training) (n=8)	Vitamin C and E Group C (pre-training (n=6)	Supplementation Group D (post-training) (n=6)
WBC (x 10^3/µl)	6.85 ± 0.40	6.80 ± 0.62	6.62 ± 0.40	6.20 ± 0.62
RBC (x 10^6/µl)	4.99 ± 0.34	5.35 ± 0.28	5.21 ± 0.28	5.24 ± 0.35
Hemoglobin (g/dl)	15.68 ± 0.78	15.73 ± 0.45	16.26 ± 0.92	16.25 ± 0.81
Hematocrit (%)	46.63 ± 2.41	46.23 ± 2.06	45.40 ± 2.61	46.35 ± 1.99
PLT (x 10^3/µl)	267.33 ± 30.64	269.17 ± 44.48	241.80 ± 15.16	245.33 ± 23.90

ased in group B. Thirty five days of VCE supplementation caused increase in cortisol levels and decrease in testosterone levels and free testosterone/cortisol ratio values. Testosterone levels and free testosterone/cortisol ratio were significantly ($p<0.05$) lower in group D than in group B although cortisol levels were higher in group D than in group B.

The mean WBC and RBC counts and hemoglobin and hematocrit values in whole blood of four groups were shown in Table 2. There was no statistical significance in WBC and RBC counts and hemoglobin and hematocrit values in the four groups.

DISCUSSION

We found that increased CK levels and free testosterone/cortisol ratio in serum were decreased by VCE supplementation although investigated free testosterone levels increased. Hence, VCE supplementation in the forced training basketball players are characterized by increased a free testosterone levels and decreased CK activities and cortisol levels. A limited number of separately VCE administrated studies in blood of forced training have been reported regarding the effects of VCE on stress marker enzymes and hormones system and LP levels [3,10,15,19]. To the best of our knowledge, the current study is the first to compare the vitamin combination with particular reference to its effects on muscle enzyme and the hormone system using levels of CK, LDH, cortisol, testosterone and hematological values in blood of forced training subjects.

The high serum activities of CK observed at baseline levels in the forced training basketball players (group B) were as high as those found directly after extreme endurance stress [19], or after resistance exercise [15]. This could be explained by the high daily degree of physical performance of the basketball layers during training, the lack of sufficient recovery time, and the specific aerobic exercise activities of this sport. The efflux of these muscle enzymes is considered to reflect a change in the normal membrane structure, induced by muscle damage, making it permeable to these molecules. In this sense, increased serum activities of CK are generally accepted as good indicator of muscle damage.

The statistically significant increase of CK at serum of forced training basketball players, and contrarily observed significant decrease of the muscle damage marker at VCE supplemented group and it might be partially explained by a protective effect of the vitamin antioxidant supplement on exercise induced damage of muscle cell membranes. Results of human studies on the effect of supplementation with antioxidant vitamin supplementation on muscle damage enzymes are controversial. Some studies favorable effects of antioxidant vitamin supplementation on these parameters after exer-

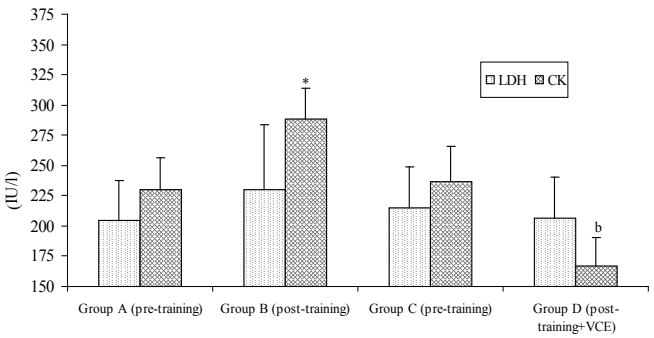

FIG. 1. LACTATE DEHYDROGENASE (LDH) AND CREATINE KINASE (CK) ENZYME ACTIVITIES IN THE SERUM OF BASKETBALL PLAYERS BEFORE AND AFTER TRAINING AND WITH AND WITHOUT VITAMIN C AND E COMBINATION SUPPLEMENTATION (MEAN±SD).

* - p<0.05 as compared with group A. [b] - p<0.01 as compared with group B.

cise [1,16], whereas others failed to demonstrate these effects [3,10,15]. Vitamin deficiency has shown to limit physical performance [8,11,16-18], but vitamin supplementation studies in humans have shown little, if any, effects on athletic performance [19]. Similar, Rokitzki and et al. [14] have shown that supplementation with vitamin E during 151 days of habitual training led to a decrease of the muscle damage marker CK in professional cyclists [14]. Contrary, Schroder et al. [15] found that statistically significant increase of LDH in serum of professional basketball players, and the contrarily observed significant decrease of this muscle damage marker after antioxidant supplementation. I did not observed statistically significant changes of LDH serum activities during the study period in each group. High statistical deviation of mean LDH serum activities might explain this fact. However, administration of VCE during the study period, might partially account for the LDH pattern observed in the supplemented group.

It is well known that skeletal muscle is regulated in part by the circulating hormonal environment, and it has been suggested that muscle adaptations may be related to changes in the anabolic and catabolic hormone profile [6]. It has also been established that circulating hormonal concentrations are influenced by the exercise [15]. In the current study, cortisol serum levels showed a similar pattern of change in both groups, with a decrease after a period of training, and a increase after VCE supplementation. In the control group (group A), the highest testosterone serum concentrations was reached immediately after exercise, as previously described by other authors [4,5]. However, in the VCE supplement group this concentration was reached at the end supplementation. As a consequence, we observed a borderline, statistically significant increase in the free testosterone/cortisol ratio in the supplemented group after training. The testosterone pattern observed in the supplement group has also been described in runners supplemented with amino acids after running exercise [7], and linked to a reduction of exercise- induced endogenous amino acid oxidation, subsequent skeletal muscle protein degradation, and prevention of testosterone muscle clearance [2]. In fact, exercise-induced free radical generation appears to lead to an increase in protein oxidation in skeletal muscle and other tissues, and VCE supplementation would reduce this process [8,11,13,17]. The protection of protein structures by antioxidants might lead to the observed effect on the anabolic/ catabolic ratio in the VCE supplement group.

CONCLUSIONS

In conclusion, we have shown that forced training is a consideration with increased CK and free testosterone in blood of basketball players. The supplementation with VCE might partially account for the hormonal and enzymes stress marker profile observed during forced training activity of basketball players.

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