Journal of Physical Activity and Hormones
Vol 3, No. 1, Ser. 9 (January 2019), 035-050

Effects of vitamin C and E supplementation on immune responses to a competition bout of sanda

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Received: 3 September 2018 / Accepted: 9 November 2018

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Abstract

Introduction: Sanda is a modern unarmed combat sport that developed from traditional wushu techniques, and primarily makes use of punching, kicking, throwing, wrestling and defensive techniques. The effects of antioxidant supplements on immune responses to this sport are unclear; thus the aim of present study was to investigate the effects of vitamin C and E supplementation on immune responses to a competition bout of sanda.

Material & Methods: Eighteen elite sanda athletes were assigned either an experimental (200 mg of vitamin C and 400 IU of vitamin E; n=9) or a placebo group (Same dosage of juice powder; n=9) using a double-blind research design. Experimental group received the antioxidant supplements for a week before the competition. Thereafter, athletes in each weight categories competed together according with the rules of International Wushu Federation (IWUF) 2016. Blood samples were taken before and immediately after the competition.

Results: The data revealed that C-reactive protein (CRP) levels were lower significantly in the experimental group than the placebo group (1.1 ± 0.6 *vs.* 1.6 ± 0.9 pg/dl; P = 0.001). For white blood cells (WBC, 8777.7 ± 100.5 vs. 9466.6 ± 972.1 n/mm^3; P = 0.4), neutrophils (50.7 ± 6.4% *vs.* 49.0 ± 7.8%; P = 0.4), monocytes (3.2 ± 1.6% *vs.* 3.6 ± 1.6%; P = 0.7), lymphocytes (43.6 ± 7.3% *vs.* 44.6 ± 7.5%; P = 0.6) and eosinophils (1.4 ± 0.5% *vs.* 1.6 ± 0.7%; P = 0.6) no significant differences were observed between the experimental and the placebo group.

Conclusions: According to the study results, vitamin C and E supplementation may have potential to enhance immune functions in sanda athletes.

Keywords: Antioxidants, Wushu, Sanda, Immune system, Inflammation

1. Introduction

Sanda is a main manifestation of Chinese martial arts, it is also called "Sanshou", and called as "Xiangbo", "Shoubo", and "Jiji", etc. Sanda, is a form of Chinese boxing. It is a martial art which was originally developed by the Chinese military based on combining the study and practices of traditional Kung fu with modern combat fighting techniques. Sanda is a highly confrontational sport. It combines full-contact kickboxing, which includes close range and rapid successive punches and kicks, with wrestling, takedowns, throws, sweeps, kick catches, and, in some competitions, even elbow and knee strikes. Its rapid development has recently led to the establishment of the Chinese National Championships, the World Championships, and the World Cup (1).

Injuries in martial arts have been reported in many previous studies. For instance, Emami et al. (2017) reported a high prevalence of injuries affecting different parts of the body in wushu athletes. They noted that about two third (65.45%) of wushu players had one or more injuries during the previous year. Severe injuries were most commonly located in the head/neck and knee/tibia areas, and inflammation was more

prevalent than the other types of injuries (2). Yiemsiri et al. (2014), also indicated that wushu is associated with higher rates of injury relative to other martial arts, because wushu is a combat sport where each player attacks his opponents to score points (3). In another study, Moghadasi et al. (2012) showed that the pro-inflammatory markers were lower in the swimmers in compare to the combat sports such as soccer and karate (4).

Several antioxidants have been introduced to protect the cells from free radicals and inflammation such as vitamins C and E, carotenoids, and flavonoids (5,6). Of course, antioxidant vitamins C and E are among the most commonly used antioxidants, as sports supplements (7). The use of antioxidant supplements can delay inflammation and oxidative stress that is caused by the effect of exercise on blood and skeletal muscle (8,9). A similar study reveals that pretreatment with vitamin C but not vitamin E can reduce C-reactive protein (CRP) among individuals with high levels of CRP (10). However, in another study, it has been concluded that 15-days vitamin C and E supplementation had no significant effect on the levels of CRP, IL-6, cortisol, lymphocyte, and lactate at rest, immediately and 1h after a session of aerobic training (11). By our knowledge, although sanda popularity growth due to the efforts for bringing wushu to the Olympic Games, no study has explored immune response to the sanda competitions and the effects of antioxidant supplements on immune responses to sanda competitions are not well known. Understanding the effect of antioxidant supplements on immune responses to sanda competition is useful for these athletes and their coaches and it may improve athlete's competitive performance. Thus the aim of present study was to investigate the effects of vitamin C and E supplementation on immune responses to a competition bout of sanda.

2. Material & Methods

Subjects

Eighteen elite sanda athletes with a minimum of 2 years competitive experience participated volunteered to participate in this study. All the subjects were in complete physical health and the exclusion criteria

included non-compliance with research intervention, and those who had consumed dietary or vitamin supplements or exogenous anabolic–androgenic steroids or other drugs, as stated in the anti-doping regulations, within the 6 weeks that preceded the study. Subjects were interviewed personally to ensure that they did not meet any obvious exclusion criteria. The subjects were informed about the experimental design and protocol and possible risks before signing the informed written consent form. The study was approved by the Marvdasht branch, Islamic Azad University Ethics Committee. The subjects were assigned either an experimental (n=9) or a placebo group (n=9) using a one-blind research design. Anthropometric characteristics of the subjects in each group are presented in the Table 1.

Table 1. Anthropometric characteristics of the subjects (Mean ± SD)

	Experimental	Placebo
Age (y)	22.6 ± 3.9	24.4 ± 1.5
Height (cm)	176.8 ± 4.4	178.6 ± 3.0
Body mass (Kg)	70.4 ± 13.1	70.3 ± 13.2
BMI ($Kg.m^{-2}$)	22.4 ± 3.8	21.9 ± 3.5

Study supplements

The subjects in the experimental group were given one tablet of 200 mg vitamin C (Modava daru, Iran) and a tablet of vitamin E 400 IU (Eurovital, Germany) for a week before the competition and the subjects in the placebo group were given capsules containing maltodextrine, noncaloric filled in empty capsules during the intervention. Both groups were advised to take the supplements and/or placebo capsules on a daily basis and after dinner. To improve the compliance, subjects were contacted more than three times a week to ensure that they had taken the supplements and/or placebo capsules which were provided on the weekly basis.

Sanda competition

After intake the supplements for a week, athletes in each weight categories [52 kg category (>48kg to ≤52kg), 6kg category (>52kg to ≤56kg), 60kg category (>56kg to ≤60kg), 65kg category (>60kg to ≤65kg), 70kg category (>65kg to ≤70kg), 75kg category (>70kg to

≤75kg), 80kg category (>75kg to ≤80kg), 85kg category (>80kg to ≤85kg), and 90kg category (>85kg to ≤90kg)] competed together according with the rules of International Wushu Federation (IWUF) 2016. The competition was not performed for 48kg category (Under ≤48kg) and over 90kg (>90kg) in this study. Athletes in each weight categories competed together and who won two out of three rounds in a bout of fight would be adopted. Each round was last two minutes and one-minute break between rounds were considered.

Biochemical analysis

Blood samples were taken from an antecubital vein in the sitting position. Six milliliters blood from a vein was taken before and immediately after the competition. Serum was separated and frozen at –20°C prior to analysis. White blood cells (WBC) differential count for neutrophils, lymphocytes, eosinophils, and monocytes was done by using automatic hematology analyzer Sysmex Kx-21. CRP levels were measured using turbidimetric assays.

Statistical analysis

Results were expressed as the mean ± SD and distributions of all variables were assessed for normality. Mean values of two groups in pre and post tests were compared by paired-samples t-test, Mann-Whitney U, Wilcoxon and independent-samples t-test for all variables. Statistical significance was accepted at $P<0.05$.

3. Results

Changes of WBC in response to vitamin C and E are presented in the Figure 1. Data indicated that WBC was increased significantly in the experimental and placebo group, however no significant differences were observed between two groups.

Research results revealed that neutrophils were decreased significantly in the placebo group and it had not significant changes in the experimental group. No significant differences were observed between two groups (Figure 2).

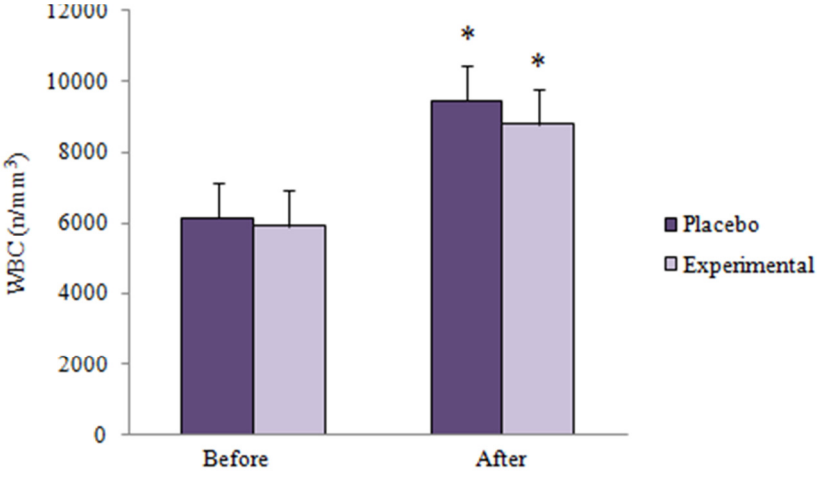

Figure 1. Changes of WBC in the experimental and placebo group
* Pre-post differences (P<0.05)

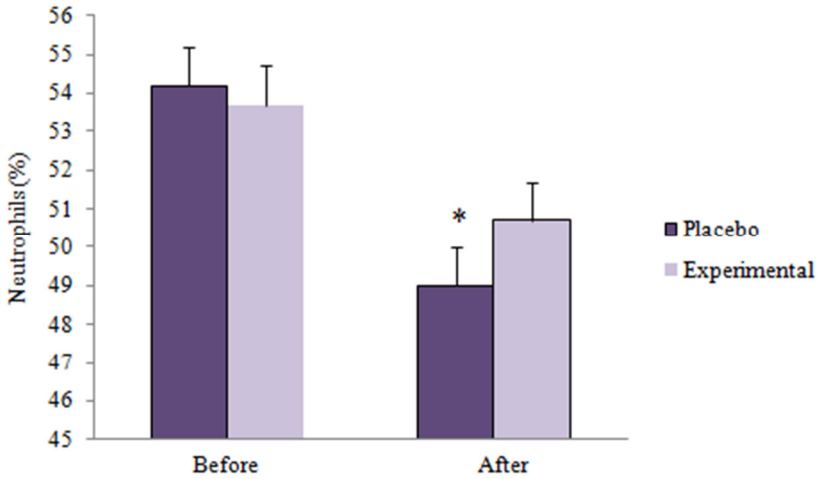

Figure 2. Changes of neutrophils in the experimental and placebo group
* Pre-post differences (P<0.05)

As shown in the Figure 3, results indicated that lymphocytes were increased significantly in the placebo group and it had not significant changes in the experimental group. No significant differences were observed between two groups.

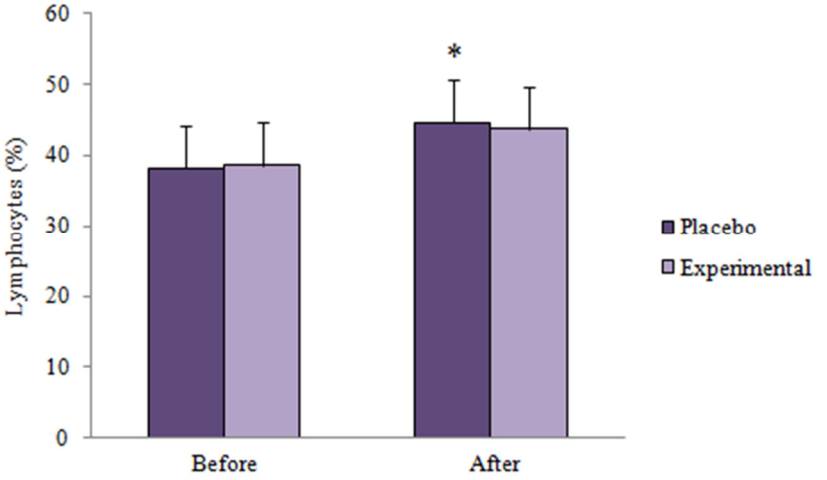

Figure 3. Changes of lymphocytes in the experimental and placebo group
* Pre-post differences ($P<0.05$)

Changes of monocytes in response to vitamin C and E are presented in the Figure 4. Data indicated that monocytes had not significant changes in response to antioxidant supplementation. No significant differences were observed between the experimental and placebo group.

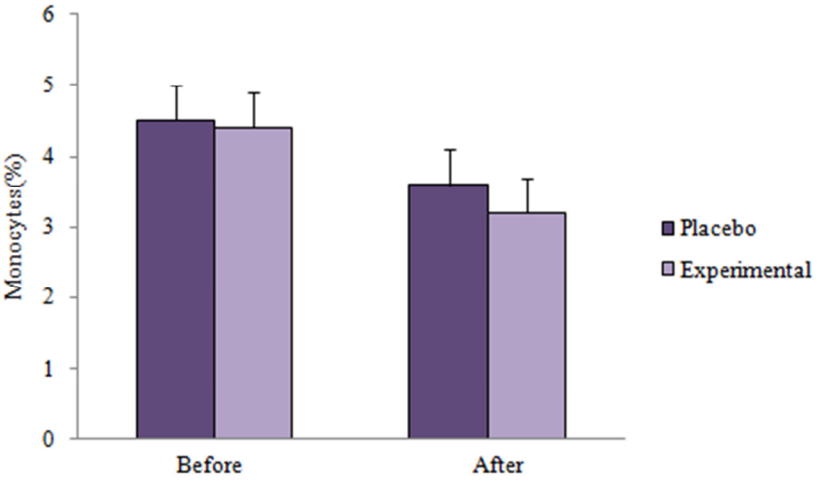

Figure 4. Changes of monocytes in the experimental and placebo group

As shown in the Figure 5, results indicated that eosinophils had not significant changes in response to vitamin C and E supplementation.

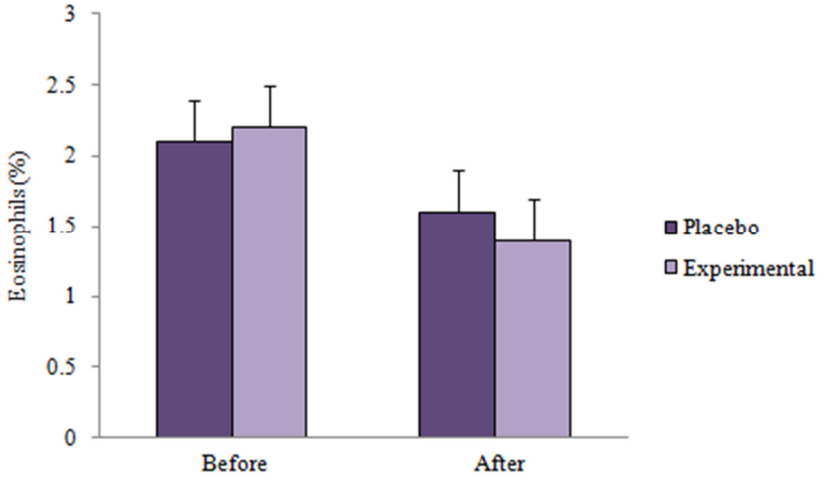

Figure 5. Changes of eosinophils in the experimental and placebo group

Research results revealed that CRP concentration decreased significantly in the experimental group in compare to the placebo group (Figure 6).

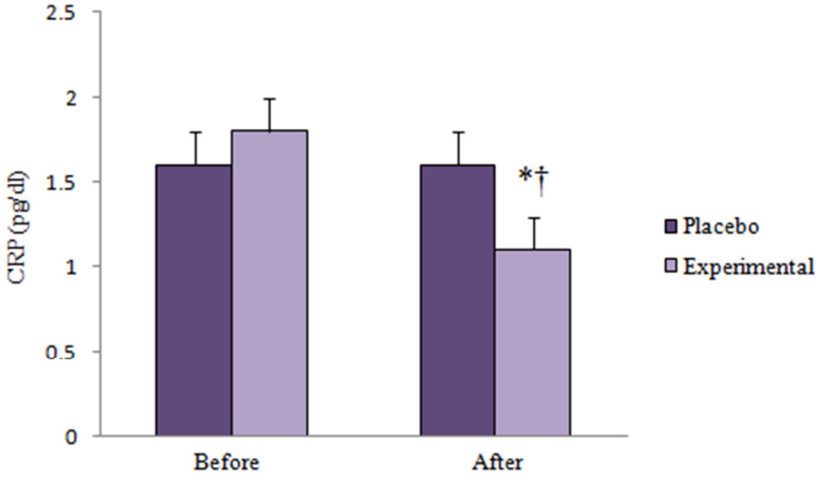

Figure 6. Changes of CRP concentration in the experimental and placebo group
* Pre-post differences (P<0.05)
† Between group differences (P<0.05)

4. Discussion

Sanda is a combat sport developed from wushu – frequently named kung-Fu – which involves striking actions, such as punches and kicks, and grappling actions, such as throwing (12). Emami et al. (2017) noted that inflammation is more prevalent than the other types of injuries among wushu athletes (2). Previous studies have shown that oral supplementation with antioxidants can attenuate the exercise-induced increase of inflammatory and pro-inflammatory markers (13-15). Antioxidant such as vitamin C and vitamin E, have been shown to attenuate oxidative stress at rest (16) as well as in response to exercise (17), the latter being a potent stimulus for increase formation of reactive oxygen species (ROS) in skeletal muscle (18). The present study was conducted to determine the effects of vitamin C and E supplementation on immune responses to a competition bout of sanda.

Moghadasi et al. (2012) showed CRP levels were lower in the non-contact sports in compare to the combat sports (4). Combat and anaerobic short term sports require much mechanical tension (19) and it seems that the contacts in such sports cause a chronic inflammation. The results of the present study in agreement with previous studies (10,20) revealed that CRP concentration were decreased significantly after the intake of vitamin C and E in the experimental group (38.8%) in compare to the placebo group. The impact of antioxidants on plasma CRP may be mediated by effects on upstream cytokines, in particular interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) which are the main inducers of the acute phase response (21). Hartel et al. (2004) found that vitamin C inhibited the lipopolysaccharide-induced IL-6 and TNF-α production, as well as IL-2 production after phorbol 12-myristate 13-acetate/ionomycin stimulation (22). Those authors suggest several potential mechanisms, including both oxidative and non-oxidative processes (22). Oxidative damage leads to an inappropriate activation of the transcription factor nuclear factor kappa beta (NFκB) and subsequently to an overexpression of inflammatory proteins (23). Vitamin C has been shown to inhibit NFκB activation (24-27). On the other hand, some studies reported that they failed to find such an effect after intake of antioxidant supplements (11,28,29). For example, Hosseini et al. (2012) indicated that 15-days

vitamin C and E supplementation had no significant effect on exercise-induced CRP (11). These discrepant results may be attributed to differences in study population and antioxidant supplements dosage.

In the present study vitamin C and E supplementation attenuates the exercise-induced increase of lymphocytes, neutrophils, monocytes and eosinophils, however there were no significant differences in WBC, lymphocytes, neutrophils, monocytes and eosinophils between groups. Hosseini et al. (2012) and Bohlooli et al. (2012) indicated that Vitamin C and Vitamin E had no significant effect on neutrophils, lymphocytes and monocytes (11,28). Laurel (1999) indicated that during exercise, lymphocytes increase less than neutrophils which lead to higher neutrophils to lymphocytes ratio during and after exercise (20). It has shown that lymphocytosis occurs during and immediately after exercise and regardless of the exercise intensity and duration (20). In this study, in line with previous studies, lymphocytes significantly increased after the sanda competition in the placebo group. 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 (11). Similar to the WBC, lymphocytes rise progressively along with the exercise intensity (20). However, unlike the leukocytosis case, here, the duration of exercise may not be a determining indicator (11). Neutrophils may be activated during exercise by a variety of factors including muscle damage, growth hormone and interleukin IL-6 (30,31). Reports of alterations in the capacity of neutrophils to generate ROS upon *in vitro* stimulation following exercise are variable. After brief, maximal exercise, some groups have reported an increase, whereas others have reported a decrease. The responses to strenuous prolonged exercise are similarly inconsistent (30,32). There is evidence from *in vitro* studies indicating that vitamin C has a strong regulatory influence on neutrophil production of ROS (33,34). Vitamin C appears to act directly by scavenging hypochlorous acid (35). Nieman et al. (1997) supplemented

runners with 1000 mg vitamin C per day for eight days prior to a 2.5-h run at 75-80% VO_{2max}. There appeared to be a small non-significant trend towards reduced *in vitro* neutrophil ROS production below pre-exercise values in the hours after exercise (36). This disparity may be due to a number of factors including differences in sampling times, the assay techniques used to measure *in vitro* neutrophil ROS production, exercise mode, and the fitness levels of participants.

5. Conclusion

In summary, vitamin C and E supplementation for a week decreases plasma levels of CRP and it can attenuate the other inflammatory markers such as lymphocytes, neutrophils, monocytes and eosinophils however no significant differences were observed for these parameters between the groups. According to the study results, vitamin C and E supplementation may have potential to enhance immune functions in sanda athletes.

6. Acknowledgment

The work was supported by grants from the Marvdasht branch, Islamic Azad University. The author gratefully acknowledges the all subjects whom cooperated in this investigation.

References

1. Jiang CY, Michael WO, Li L. Determination of biomechanical differences between elite and Novice san Shou female athletes. J Exerc Sci Fit 2013; 11: 25-28.

2. Emami F1, Bahremand M, Z Rojhani-Shirazi Z. Prevalence of Musculoskeletal Injuries in Shiraz Male Wushu Players: A Cross Sectional Study. J Rehab Sci Res 2017; 4: 6-9.

3. Yiemsiri P, Wanawan A. Prevalence of injuries in Wushu competition during the 1st Asian Martial Arts Games 2009. J Med Assoc Thai 2014; 97(Suppl 2): S9-S13.

4. Moghadasi M, Karami Tirehshabankare M, Golnary T, Parsa B, Nematollahzadeh M. Resting C-reactive protein levels in male and female athletes. Medicina dello Sport 2012; 65: 57-62.

5. Janero DR. Therapeutic potential of vitamin E in the pathogenesis of spontaneous atherosclerosis. Free Radic Biol Med 1991; 11: 129-144.

6. Packer L. Protective role of vitamin E in biological systems. Am J Clin Nutr 1991; 53: 1050S–1055S.

7. McGinley C, Shafat A, Donnelly AE. Does antioxidant vitamin supplementation protect against muscle damage? Sports Med 2009; 39: 1011-1032.

8. Sen CK, Atalay M, Hanninen O. Exercise-induced oxidative stress: glutathione supplementation and deficiency. J Appl Physiol 1994; 77: 2177-2187.

9. Ashton T, Young IS, Peters JR, Jones E, Jackson SK, Davies B, et al. Electron spin resonance spectroscopy, exercise, and oxidative stress: an ascorbic acid intervention study. J Appl Physiol 1999; 87: 2032-2036.

10. Block G, Jensen CD, Dalvi TB, Norkus EP, Hudes M, Crawford PB, et al. Vitamin C treatment reduces elevated C-reactive protein. Free Radic Biol Med 2009; 46: 70-77.

11. Hosseini SA, Hosseini ZS, Noura M, Keikhosravi F, Hassanpour G, Azarbayjani MA, et al. The effect of vitamin C and E supplementation on CRP, IL- 6, lymphocyte, cortisol and lactate response following one aerobic training session. J Pharmaceutic Health Sci 2012; 1: 29-44.

12. Zeng YJ, Zhang L, Zhou M. On wushu sanshou and wushu sanda. J Wuhan Institute Physic Edu 2009; 43: 69-73.

13. Vassilakopoulos T, Karatza MH, Katsaounou P, Kollintza A, Zakynthinos S, Roussos C. Antioxidants attenuate the plasma cytokine response to exercise in humans. J Appl Physiol 2003; 94:1025-1032.

14. Fischer CP, Hiscock NJ, Penkowa M, Basu S, Vessby B, Kallner A, et al. Supplementation with vitamins C and E inhibits the release of interleukin-6 from contracting human skeletal muscle. J Physiol 2004; 558: 633-645.

15. Popovic LM, Mitic NR, Miric D, Bisevac B, Miric M, Popovic B. Influence of vitamin C supplementation on oxidative stress and neutrophil inflammatory response in acute and regular exercise. Oxid Med Cell Longev 2015; 2015: 295497.

16. Huang HY, Appel LJ, Croft KD, Miller ER 3rd, Mori TA, Puddey IB. Effects of vitamin C and vitamin E on in vivo lipid peroxidation: results of a randomized controlled trial. Am J Clin Nutr 2002; 76: 549-555.

17. Kanter MM, Nolte LA, Holloszy JO. Effects of an antioxidant vitamin mixture on lipid peroxidation at rest and postexercise. J Appl Physiol 1993; 74: 965-969.

18. Davies KJ, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 1982; 107: 1198-1205.

19. Mackinnon LT. Advances in exercise immunology: Human Kinetics, 1999.

20. Laurel T. Advance in exercise immunology, human kinetics, 1999.

21. Baumann H, Goldie J. The acute phase response. Immunol Today 1994; 15: 74-80.

22. Hartel C, Strunk T, Bucsky P, Schultz C. Effects of vitamin C on intracytoplasmic cytokine production in human whole blood monocytes and lymphocytes. Cytokine 2004; 27: 101-106.

23. Baeuerle PA, Henkel T. Function and activation of NFκB in the immune system. Annu Rev Immunol 1994; 12: 141-179.

24. Bowie AG, O'Neill LAJ. Vitamin C inhibits NF-κB activation by TNF via the activation of p38 mitogen-activated protein kinase. J Immunol 2000; 165: 7180-7188.

25. Carcamo M, Pedraza A, Borquez-Ojeda O, Golde DW. Vitamin C suppresses TNFα-induced NFκB activation by inhibiting IκBα phosphorylation. Biochemistry 2002; 41: 12995-13002.

26. Campbell JD, Cole M, Bunditrutavorn B, Vella AT. Ascorbic acid is a potent inhibitor of various forms of T cell apoptosis. Cell Immunol 1999; 194: 1-5.

27. Perez-Cruz I, Carcamo JM, Golde DW. Vitamin C inhibits FAS-induced apoptosis in monocytes and U937 cells. Blood 2003; 102: 336-343.

28. Bohlooli S, Rahmani-Nia F, Babaei P, Nakhostin-Roohi B. Influence of vitamin C moderate dose supplementation on exercise-induced lipid peroxidation, muscle damage and inflammation. Medicina dello Sport 2012; 65; 187-197.

29. Kashef M. Effect of vitamin E supplementation on delayed onset muscle soreness in young men. J Physic Act Horm 2018; 2: 15-28.

30. Peake JM. Exercise-induced alterations in neutrophil degranulation and respiratory burst activity: possible mechanisms of action. Exerc Immunol Rev 2002; 8: 49-100.

31. Suzuki K, Totsuka M, Nakaji S, Yamada M, Kudoh S, Liu Q, et al. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. J Appl Physiol 1999; 86: 1360-1367.

32. Suzuki K, Sato H, Kikuchi T, Abe T, Nakaji S, Sugawara K, et al. Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. J Appl Physiol 1996 81: 1213-1222.

33. Anderson R, Smit M, Joone G, van Staden A. Vitamin C and cellular immune functions. Ann NY Acad Sci 1989; 587: 33-48.

34. Dwenger A, Funck M, Lueken B, Schweitzer G, Lehmann U. Effect of ascorbic acid on neutrophil functions and hypoxanthine/xanthine oxidase-generated, oxygenderived radicals. Eur J Clin Chem Clin Biochem 1992; 30: 187-191.

35. Anderson R, Lukey P. A biological role for ascorbate in the selective neutralization of extracellular phagocyte-derived oxidants. Ann N Y Acad Sci 1987; 498: 229-247.

36. Nieman D, Henson D, Butterworth D, Warren B, Davis J, Fagoaga O, et al. Vitamin C supplementation does not alter the immune response to 2.5 hours of running. Int J Sport Nutr 1997; 7: 173-184.