

# The Effect of Endurance Swimming Plus Vitamin C Supplement on Oxidative Stress and Muscles Damage Indices in Male Wistar Rats

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## Abstract

**Background:** Research suggests that the effects of endurance training and supplementation with vitamin C on oxidative stress and muscle damage is associated with conflicting results that can be affected by the level of exercise and the amount and type of antioxidant supplements consumed.

**Objectives:** The aim of the present research was to study the effect of endurance swimming training with the consumption of a vitamin C supplement on indices of oxidative stress and muscle damage in male Wistar rats.

**Materials and Methods:** Twenty-four male Wistar rats with body weights of  $275 \pm 25$  g were randomly divided into four groups of six: training (T), training with vitamin C (T+VC), control (C), and control with vitamin C (C+VC). Training groups swam for one hour per day and five days per week for 10 weeks. A vitamin C supplement 100 mg/kg b w solution with water rats and started one week before the training protocol began and continued to the end of the tenth week. To indicate the variables of catalase (CAT), malondialdehyde (MDA), total antioxidant capacity (TAC), uric acid (UA), lactate dehydrogenase (LDH), and creatine kinase (CK), blood sampling was done on vena cava one day after the end of the training protocol. The results were analyzed using the one-way ANOVA followed by a Tukey test. The significance level was less than 0.05.

**Results:** The results of this research indicated that the T+VC group showed a significant reduction in the level of MDA compared with the C group ( $P=0.008$ ). A significant increase in the level of TAC was observed in the C+VC group compared with the T group ( $P=0.03$ ). Both the T and T+VC groups indicated significant increases in the levels of LDH and CK compared with both the C and C+VC groups ( $P=0.001$ ).

**Conclusions:** In sum, the results indicate that the consumption of vitamin C can decrease the lipid peroxidation and increase the level of TAC, and is ineffective on enzyme and non-enzyme antioxidants and muscle damage.

**Keywords:** Endurance Swimming, Muscle Damage, Oxidative Stress, Vitamin C

## 1. Background

Oxygen is vital for all aerobic reactions. Gerschman et al. (1954) stated that the damaging effects of oxygen are associated with the production of oxygen free radicals (1). Proportionate with time and distance during exercise, oxygen increases 10 to 20 times and oxygen flow increases 100 times to the active muscles and their metabolism increases up to 200 times (2). Free radicals are very active and react with other molecules to enable electrons to reach a stable state. Oxidative stress is defined as disturbing the balance between the production of free radicals and antioxidant defenses (3). Overall, antioxidant defenses can be divided into two categories: enzymatic and non-enzymatic. Uric acid is a non-enzymatic antioxidant and its function as an important cleaner of free radicals in the plasma has been highlighted (3). Catalase (CAT) is considered to be an antioxidant which can neutralize their oxides through the analysis of free radicals and reduction of the risk of hy-

droxyl radical's formation. Malondialdehyde is the main product of polyunsaturated fatty acids destruction which is considered to be an indicator of oxidative damage to lipids, such as membrane phospholipids (3). Total antioxidant capacity (TAC) indicates the total number of antioxidants in the body (4-7). During intense endurance activities, TAC is reduced to reflect the weakening role of such activities in the antioxidant defense system (8). Intense exercise can lead to muscle and cell damage. In fact, as a result of the disruption of the sarcomere and muscle cell membrane and extracellular fluid leakage, increases the concentration of enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH) in the serum (8).

CK and LDH, which are involved in the anaerobic path of ATP production, are known as an indicator of oxidative stress (9). Vitamin C (ascorbic acid) is the most important water-soluble antioxidant that is located in the cytosol and extracellular fluid. It helps to clean  $H_2O_2$ , ROS, and OH $^-$  formed in aquatic environments such as plasma (10). Vita-

min C can hunt free radicals to reduce lipid peroxidation. In theory, benefiting from the antioxidant defense that results from physical activity and vitamin C intake can prevent the damage caused by oxidative stress on the body's vital tissues (11).

Gupta (2009) observed a significant increase in the concentration of CAT blood serum of subjects after two months of taking vitamin C supplements (500 mg/d) and the exhaustive training, than before supplementation. The content of MDA had a significant decrease (12).

Lekhi (2007) showed a significant increase in the content of MDA and uric acid in serum after an exhaustive endurance exercise, but subjects had a significantly lower level of catalase than the control group (13). Babai (2009) divided 24 healthy non-athlete males into three groups of eight: group 1 was the placebo group, group 2 took 500 mg of vitamin C, and group 3 took 1000 mg of vitamin C. After 30 minutes of exercise at 75%  $\text{VO}_2$  max, groups 2 and 3 had significantly decreased serum MDA and a significant increase in serum TAC and CK after exercise compared to the placebo group (14).

Nakhostin-Roohi et al. (2007) divided 16 healthy non-athlete males into two groups: the placebo group and a group who took a vitamin C supplement. Participants ran 30 minutes by 75% volume  $\text{O}_2$  maximum. The level of MDA after the activity was higher in the placebo group. CK in both groups increased, but it was reduced in the supplement group 24 hours after the physical activity (15).

Mastaloudis et al. (2006) examined the effect of antioxidant supplements on markers of muscle damage in ultramarathon runners. They showed that plasma markers of muscle damage after endurance exercise increase and are not affected by the antioxidant supplements (16). Traber et al. (2006) studied 22 male and female runners who took the vitamin supplement and participated in a marathon race and showed that taking vitamin supplements prevents oxidative stress injury and lipid peroxidation, but has no effect on muscle damage (17).

Cavas (2004) reported different results and expressed that taking antioxidant supplements reduces oxidative stress, CK, and LDH (18). Raphael (2007) observed the reduction of the CK level due to the use of antioxidant supplements and endurance exercise (19).

Research suggests that the effects of endurance training and supplementation with vitamin C on oxidative stress and muscle damage is associated with conflicting results that can be affected by the level of exercise and the amount and type of antioxidant supplements consumed.

## 2. Objectives

Since the body's antioxidant response after exercise of swimming in interaction with vitamin C has not been checked, the aim of this study was to investigate the interaction of vitamin C and submaximal swimming on antioxidant levels of catalase, uric acid, total antioxidant capacity, lipid peroxidation, and muscle damage.

## 3. Materials and Methods

This is an applied experimental study that was performed in 2015 at the Hamadan University of Medical Sciences (Iran). Subjects of the study were 24 male Wistar rats weighing in the range of 250 to 300 g. Animals were kept in the standard conditions of 12 hours of light, 12 hours of darkness, temperature of  $21 \pm 1^\circ\text{C}$ , and humidity of  $25 \pm 5$  percent. Food and water were freely provided. The rats were randomly divided into four experimental groups ( $n = 6$ ): swimming training (T), swimming training with vitamin C (T + VC), control (C), and control with vitamin C (C + VC). The rats' swimming pool consisted of a plastic tub sized  $60 \times 60 \times 100$  cm for each training group. Water temperature was  $32 \pm 2^\circ\text{C}$ .

### 3.1. The Training Protocol

One week of swimming training was considered for rats adapt to their environment. Thus, the first session began with 20 minutes of swimming, the second session consisted of 40 minutes of swimming, and the third session consisted of 60 minutes of swimming. The swimming training protocol consisted of ten weeks, five days per week, and one hour per session.

A vitamin C supplement powder was purchased from Sigma company and was solved in the ratio 100 mg/kg in body weight in water. The supplement solution was provided daily and started with the compatibility program and continued until the end of the tenth week.

A day after the end of the protocol, the rats were anesthetized by pentobarbital sodium gas (50 - 60 mg/kg). In order to estimate the parameters of catalase (CAT), malondyaldehyde (MDA), total antioxidant capacity (TAC), uric acid (UA), lactate dehydrogenase (LDH), and creatine kinase (CK), blood samples were collected from the inferior vena cava of rats and the serum was separated by centrifugation. The serum was poured into micro tubes and was maintained at  $-20^\circ\text{C}$  until the end analysis. Serum uric acid was measured based on uric acid oxidation by the enzyme (20).

b) The MDA levels were measured by spectrometer according to a previous published method (21). Under this

method is, reaction tiobarbituric acid (TBA) with Lipid peroxidation. This acid breaks down molecule lipid peroxidation in MDA, and then MDA reacts with TBA to produce a substance that is measured in fluorescent spectrophotometry (21).

c) Serum catalase levels were measured by a spectrophotometer. The measurement was based on the decomposition of  $H_2O_2$  at a wavelength of 240 nm and a temperature of 20°C (21).

d) Determination of TAC in serum was done by FRAP test. In this method, the ability of serum is checked at the reduction of ferric ions. With revival ferric ion and conversion it to ferrous ions at acidic pH and presence of specific reagent, blue hydroelectric complex will be created which is measured at length wave 593 nm via spectrophotometer (20).

e) Serum CK and LDH were determined in the chemical colorimetric method according to manufacture protocol (Pars Azmoon, Tehran, Iran).

### 3.2. Statistical Analysis

Results were presented as mean  $\pm$  SEM. The difference between the mean was determined using one-way ANOVA, and Tukey post hoc test was used to determine the differences between the groups.  $P < 0.05$  was used as the level of significance of the mean difference.

## 4. Results

Serum uric acid levels in the training and control groups who had taken the supplement were decreased compared to the non-supplement groups, but this difference was not statistically significant (Figure 1A).

Catalase levels in the exercise group increased compared with the other groups, but this increase was not statistically significant (Figure 1B).

Statistical analysis showed that MDA levels in the training groups fell, but the decrease in the T + VC group was significant compared with the C group ( $P = 0.008$ ) (Figure 1C).

The statistical results showed that TAC levels were significantly increased in the C + VC group compared to the T group ( $P = 0.03$ ) (Figure 1D).

Statistical analysis showed that the levels of CK in the T group increased significantly ( $P < 0.001$ ) compared to the C group and the C + VC group. The values in the T + VC group had a significant increase ( $P < 0.001$ ) compared with the C group and the C + VC group.

The statistical results showed that LDH levels in the T + VC group increased significantly compared to that in the T group ( $P < 0.001$ ) and the C group ( $P < 0.001$ ). This result showed as a significant increase in the T + VC group

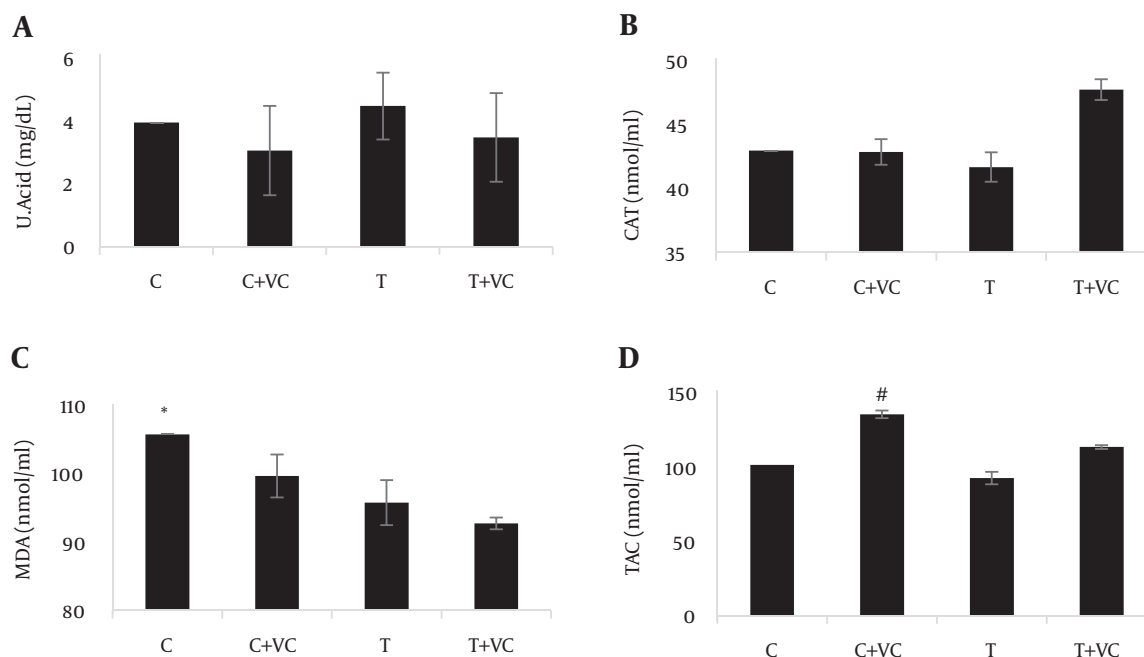
compared with the C group ( $P < 0.001$ ) as well as the C+VC group ( $P < 0.001$ ).

## 5. Discussion

Analyzing the results of serum uric acid showed that the groups' vitamin C supplement not only did not have higher uric acid levels, but also showed a lower level compared to the non-supplement groups. Nieman's (2002) study on male runners in which participants were treated with 1500 mg vitamin C for a week found that levels of uric acid decreased in the supplementation group compared to the control group (22). Also, in the study of Lekhi (2007), elite cyclists who did not take antioxidant supplements showed a significant increase in plasma uric acid content (13). The production of uric acid is a defense mechanism against oxidative stress and a useful response to maximal physical activity; it can be released into the muscles and prevent lipid peroxidation and vitamin C oxidation. In fact, uric acid is one of the most important antioxidants that has independent effects on  $H_2O_2$ , HOCl, and peroxide nitrite (23). On the other hand, vitamin C acts as an electron donor to vitamin E when oxidative stress occurs in the cell membrane (24). Therefore, it seems that one of the reasons for the reduction in plasma uric acid levels is the deletion of free radicals by vitamin C antioxidant activity. On the other hand, training intensity of submaximal swimming leads to the reduction of serum content of uric acid in the groups receiving the supplement.

Results of the plasma level of catalase suggested that the T + VC group had an increase in the plasma concentration of this antioxidant enzyme compared with the other three groups; however, this increase was not statistically significant. Moreover, the catalase serum content of the C + VC group, showed no significant difference compared to the C group. This result was consistent with that of Zoppi (2006) and Machefer (2007). In both studies, the concentration of catalase in the supplement group was not significantly different from its concentration in the control group (25, 26). In a study by Gupta (2009), the group receiving the vitamin C supplement, showed a significant increase in their serum CAT after doing exhaustive running compared to the time before taking the supplement (12). However, Sari Sarraf (2013) observed a significant decrease in the content of serum CAT of the supplement group after 14 days of supplementation and speed skating (27).

The catalase can deal with free radicals in two ways: by catalyzing the decomposition of  $H_2O_2$  to water and oxygen or by preventing the formation of  $H_2O_2$  (28). An overlap exists between the performance of CAT and glutathione peroxidase (GPx). Compared to CAT glutathione peroxidase plays a more active role in removing free radicals in low

**Figure 1.** Comparison Basal Levels of Index of the Oxidative Stress in the Control and Trained Groups

A, U. Acid (Uric Acid); B, CAT (Catalase); C, MDA (Malondialdehyde); D, TAC (Total antioxidant capacity). Groups - C, control group; C+VC, control with vitamin C; T, training; T + VC, training with vitamin C. \*: Significant reduction level of MDA in T+VC group compared to C group ( $P=0.008$ ). #: Significant increase level of TAC in C + VC group compared to T group ( $P=0.03$ ).

**Table 1.** Basal Levels of Index of the LDH and CK in the Control and Trained Groups<sup>a</sup>

Factors	Groups			
	C	C+VC	T	T+VC
LDH (U/L)	1393 ± 46.34	475.66 ± 86.85 <sup>b,c</sup>	1960.4 ± 261.55 <sup>d</sup>	2935.4 ± 167.35 <sup>e,f</sup>
CK (U/L)	581 ± 179.97	173 ± 46.34 <sup>b,c</sup>	1418 ± 95.75	1458.4 ± 117.06 <sup>e</sup>

<sup>a</sup> Values are expressed as Mean ± SEM.

<sup>b</sup> Comparison of C + VC group with T group.

<sup>c</sup> Comparison of C + VC group with T + VC group.

<sup>d</sup> Comparison of T group with C group.

<sup>e</sup> Comparison of T + VC group with C group.

<sup>f</sup> Comparison of T + VC group with T group, ( $P < 0.001$  for all).

Abbreviations: C + VC, control with vitamin C; CK, creatine kinase; Groups - C, control; LDH, lactate dehydrogenase; T, training; T + VC, training with vitamin C.

concentrations of  $H_2O_2$ ; however, when the concentration of  $H_2O_2$  in the cell increases, catalase comes in to play (29). De-castro (2009) (who studied sedentary elderly men) and Daud (2006) (who studied trained young men) stated that the intensity of activity more than 60 percent of maximum heart rate (anaerobic) reduces the activity of this enzyme (30, 31). It was also found that muscle fibers with low oxidative capacity have lower levels of CAT activity compared to oxidative fibers (29). Catalase activity of marathon run-

ners is two times more than that of sprinters. Some of the possible reasons for these two issues can be a) the nature of submaximal aerobic exercise (less than 60% HRmax), b) increased oxygen consumption (more  $VO_2max$ ) in endurance activities, and c) the fact that slow-twitch fibers are more summoned. In general, the reasons of no change or decrease in catalase activity include decreased production of hydrogen peroxide due to the antioxidant activity of vitamin C; no need to increase the activity of catalase due

to the activity of GPx at low concentrations of H<sub>2</sub>O<sub>2</sub>; or a combination of the above two factors (29).

After examining the serum content of MDA, it was observed that there was a significant decrease in the concentration of this index in the T + VC group compared to the C group. However, there was no significant difference in MDA levels between experimental groups. In studies done by Gholami (2014), Babaei (2009), and Popovic (2015), there was a significant reduction in MDA serum concentration of the training group treated with supplementation (14, 32). However, Goldfarb (2005) did not observe any significant difference in the MDA level of the exercise group receiving vitamin C supplementation and the control group (33). A variety of sports activities led to oxidative stress and oxidative damage to lipids, and subsequent production of lipid peroxidation through increased production of reactive species (34). Long-term exercise decreases blood cholesterol and plasma LDL. Therefore, part of the reduction in the levels of MDA may be due to reduced availability of fatty acids (35). In general, three factors are involved in this reduction: a) a decrease in the production of free radicals in the body, b) an increase in the activity of antioxidant enzymes, and c) a balancing of oxidants against antioxidants in the body. In addition, by reviving free radicals and turning them into ascorbic acid radicals, vitamin C prevents oxidative stress and MDA increase, resulting in a negative correlation between plasma ascorbic acid (PAA) and MDA (36). Therefore, taking this antioxidant supplement significantly inhibits lipid oxidation in the training group compared to the control group.

As shown in the results, supplementation of vitamin C in the control group resulted in a significant increase in the content of TAC compared to the no-supplement training group. As expected, this increase agreed with the results of Sari-Sarraf (2013), Babaei (2009), and Nour-shahi (2012) (14, 27, 37). Vitamin C supplementation increased the levels of serum ascorbic acid and subsequently increased TAC (38). Taking a vitamin C supplement can lead to an increase in TAC by donating electrons to vitamin E and restoring this fat-soluble antioxidant. This antioxidant action of vitamin C occurs following oxidative stress and prevents further lipid peroxidation on the surface of the cell membrane (24).

The statistical results suggested that both training groups showed a significant increase in the serum level of CK compared to both control groups. Rouhi (2008), Mastaloudis (2006), and Traber (2006), also reported the inefficiency of antioxidant supplements in reducing the level of CK (15-17), while Cava (2003) and Raphael (2007) observed a significant reduction in the supplement receiving groups (18, 19). Normally, creatine kinase does not enter the extracellular space unless there is harm to

the sarcolemma. Creatine kinase is one of the enzymes of the phosphagen system that is important for energy metabolism in most cells of the body, especially in muscle and brain cells (39). Usually, this enzyme is a very strong indicator for evaluating muscle damage. Many researchers have stated that taking antioxidant supplements reduces oxidative stress and prevents muscle damage by invigorating the body's antioxidant defenses (40). During swimming and when the energy devices (including phosphagen, and aerobic and anaerobic glycolysis) are active, an increase in the concentrations of creatine kinase for producing ATP in training groups seems natural. As observed, supplementation did not result in an increase in the TAC of training groups; therefore, it failed to prevent muscle damage.

Results showed that both experimental groups showed a significant increase in the level of lactate dehydrogenase compared to both control groups. Moreover, the T group had a significant reduction in LDH levels compared to the T + VC group. Traber (2006) and Mastaloudis (2006) also observed an increase in LDH levels in the training groups with and without supplement (16, 17). However, Cava and Raphael reported the reduction of LDH levels as a result of taking antioxidant supplementation (18, 19). The LDH enzyme is widely distributed in body tissues and its high concentration is seen in the liver, myocardium, kidney, skeletal muscles, red blood cells, and other tissues (41). The symptoms of muscle damage include the emergence of intramuscular enzymes, such as CK and LDH, in the blood (42). One-hour activity of swimming led to increased muscle damage in the training groups; taking antioxidant supplements did not reduce the damage. It seems that the dose of vitamin C has been unable to increase TAC.

### 5.1. Conclusion

Overall, it can be said that swimming training with moderate intensity, along with taking vitamin C, can inhibit lipid peroxidation, and consumption of vitamin C can increase TAC of the control group. However, it has no effect on CAT and uric acid levels. Moreover, the exercise groups had more muscle damage compared to the control groups.

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