

## Effects of Swim Training and Vitamin C Supplementation on the Antioxidant System Following Exhaustive Exercise Stress

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### Abstract

This study was intended to investigate the effects of regular swimming exercise and vitamin C supplementation on the antioxidant system following exercise stress. For the swimming exercise experiment, a swimming adaptation exercise of 1 week was given to a group of 6-week-old mice. Following this, a swimming exercise for 8 weeks was conducted. The experimental group was divided into 3: a control group (C), a swimming exercise trained group (T), and a group of swimming + vitamin C supplementation (TC: vitamin supplementation: 1.3 mg/100 g diet). After the swimming exercise, these group were further divided into those that had received the exercise stress for 2 hours and those that had not experienced exercise stress group. Then, the activities of the superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) concentrations were measured. There was a lower weight increase in the T and TC groups than in the C group, and there was no significant difference between T and TC group. When exercise stress was not experienced, the activity of SOD was significantly increased in the TC group than in the T group, but there was no significant difference between C and T groups. The groups that had experienced a 2-hour exercise stress showed the SOD activity levels according to the following order, C < T < TC, with a significant difference between the three groups ( $p < 0.05$ ). There was no difference in MDA concentration amongst the experimental groups in non-exercise stress group. As well, there was no differences in MDA concentration between the C group and T group in the 2 hour exercise stress group. However, the TC group showed a MDA concentration level significantly lower than that of the T group. A significant increase in MDA concentration was observed in C group, when exercise stress was provided with no significant difference in the T and TC groups. As a result, regular exercise and vitamin C supplementation can be considered important in controlling the formation of lipid peroxides in exercise stress.

**Key words:** swimming exercise, vitamin C supplementation, exhaustive exercise stress GSH-Px, SOD, MDA

### INTRODUCTION

Regular aerobic activity has long been known to optimize the immune system of the body, in addition activating the antioxidant system (1). In fact, many studies have been carried out on the influence of regular aerobic exercises on the antioxidant system (2). These studies have recently reported, for example, that regular training helps not only strengthen the antioxidant system but also optimize the immune system. As such, these studies have further revealed that the groups with aerobic training show more activation of the antioxidant enzyme system than a typical control group (3), regular training results in the relative reduction of lipid peroxides (2), and the antioxidant enzyme system is activated by vitamin supplementation (4).

It has been known, nevertheless, that these effective

results can be successfully achieved only when aerobic exercise is performed properly and regularly. By contrast, rather exercising vigorously and suddenly causes the body to be harmfully stressed (5). It has been reported that the increased oxygen intake during such exercising increases the formation of ROS (reactive oxygen species), including superoxides and hydroxy radicals in the metabolic process within the cells (6,7).

In addition, sudden vigorous exercise stresses the heart, skeleton muscle and other body tissues, with damage varying in accordance with the training level. It has been reported that generally less the experience one has in training, higher the stress level (7). It has been also reported that the harmfulness of active oxygen can be minimized when the antioxidant activity is improved by vitamin C supplementation (8). In fact, Choi et al. (9) reported that the formation of lipid peroxides is reduced

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with exercise intensity levels of 85%HR max when a multiple vitamin containing vitamin C and E is supplemented.

Accordingly, the purpose of this study is to analyze the effects of swim training and vitamin C supplementation on antioxidant enzyme activity and peroxidation, and to comparatively observe their effects following compulsory swim stress, thereby examining the effects of regular training and vitamin C supplementation on the antioxidant system and lipid peroxide after non exercise stress versus exhaustive exercise stress.

## MATERIALS AND METHODS

### Experimental animal and diet

Male BALB/c mice were bred in the Animal Unit under controlled conditions (22~24°C, RH 50~60%) including the provision of food and water *ad libitum*. Animals were 6 weeks old at study commencement and were fed an AIN-93 diet for the 10-week study period. Diet composition is shown in Table 1. The TC group diet was supplemented with vitamin C (1.3 mg/100 g diet). Animals had access to distilled deionized water *ad libitum*. The daily feed intake and weekly body weight gain were routinely recorded throughout the experimental period. Mice were divided into a control group (C), a swimming exercise trained group (T), and one being trained and provided with the vitamin C supplementation (CT). All groups were further subdivided equally into two periods based on whether the mice were studied at rest or immediately after a 2-hour acute bout of exercise. The trained groups swam in a tub measuring 63 cm × 40 cm × 18 cm deep for 30 minutes/day during the first week, and during subsequent weeks, this was extended by 10 minutes/week up to 60 min/day. The size of the tub ensured that the mice would swim freely and not float

**Table 1.** Diet composition of experimental diet (g/kg diet)

Composition	Groups <sup>1)</sup>	
	C, T	TC
Casein	400.0	400.0
Cornstarch	272.4	272.4
Dextrinized cornstarch	132.0	132.0
Soybean oil	70.0	70.0
Fiber	50.0	50.0
Mineral mix <sup>2)</sup>	35.0	35.0
Vitamin mix <sup>2)</sup>	10.0	10.0
L-Cystine	3.0	3.0
Choline bitartrate	2.5	2.5
Tert-butylhydroquinone	0.014	0.014
Vitamin C (mg) supplementation	13 mg	

<sup>1)</sup>C: Control group, T: Swimming trained group, TC: Swimming trained + vitamin C supplemented group.

<sup>2)</sup>AIN-93.

passively. The water temperature was kept at 27~30°C. At the end of the training period, specifically 48 h after the last training session, the animals were anaesthetized with CO<sub>2</sub> and sacrificed to exclude training effect.

### Antioxidant enzyme and MDA concentration analysis

The plasma SOD activity was measured in compliance with the methods of Marklund and Marklund (10). Briefly, SOD was detected on the basis of its ability to inhibit superoxide-mediated reduction. One unit was defined as the amount of enzymes that inhibited the oxidation of pyrogallol. And GSH-Px analysis was detected with coupled enzyme procedure by following the modified method of Paglia et al. (11) and Deagen et al. (12). Plasma MDA was assayed according to the fluorometric method described by Buckingham (13). MDA reacts with TBA to form absorption adduct with a maximum absorption at 532 nm.

### Statistical analysis

Statistical analysis was done using SPSS program. The results were presented as mean ± SEM and the differences among experimental groups were analyzed by one-way analysis of variance (ANOVA) with Duncan's multiple range test at p < 0.05. The mean difference between non stress group and 2-hour stress group was analyzed using Student *t*-test at p < 0.05.

## RESULTS AND DISCUSSION

### Body weight and food intake

As shown in Table 2, the body weights of the animals were 23.3 ± 0.75 g, 23.0 ± 1.04 g, 24.0 ± 0.82 g at 6 weeks old. After 10 weeks of swim training (at 16 weeks old), their average body weights were 32.6 ± 1.52 g for the untrained controls, 28.1 ± 1.37 g for T, and 28.4 ± 1.42 g for CT. The T group had a significantly lower body weight than the C group after swim training (p < 0.05). However, there was no significant difference between the T and CT groups. Food intakes of the animal showed no differences among the experimental group.

**Table 2.** Body weight and food intake in the experimental group (g)

Groups <sup>1)</sup>	Body weight		Food intake
	Initial	Final	
C	23.3 ± 0.75	32.6 ± 1.52 <sup>a2)</sup>	4.72 ± 0.35
T	23.0 ± 1.04	28.1 ± 1.37 <sup>b</sup>	5.12 ± 0.53
TC	24.0 ± 0.82	28.4 ± 1.42 <sup>b</sup>	5.36 ± 0.32

<sup>1)</sup>Groups are the same as Table 1.

<sup>2)</sup>Values with different superscript letters within a column are significantly different from each other group at p < 0.05.

**Table 3.** Antioxidant enzyme activities in the experimental group

Groups <sup>1)</sup>	GSH-Px (U/mg prot)		<i>t</i> -test	SOD (U/mg prot)		<i>t</i> -test
	Non exercise stress	2 hr exercise stress		Non exercise stress	2 hr exercise stress	
C	3.45 ± 0.15 <sup>b2)</sup>	3.75 ± 0.32 <sup>b</sup>	NS <sup>3)</sup>	4.62 ± 0.23 <sup>b</sup>	3.52 ± 0.41 <sup>c</sup>	*
T	4.52 ± 0.14 <sup>a</sup>	5.28 ± 0.42 <sup>a</sup>	NS	4.28 ± 0.38 <sup>b</sup>	5.13 ± 0.30 <sup>b</sup>	NS
TC	4.65 ± 0.21 <sup>a</sup>	5.23 ± 0.21 <sup>a</sup>	NS	5.92 ± 0.47 <sup>a</sup>	6.72 ± 0.52 <sup>a</sup>	NS

<sup>1)</sup>Groups are the same as Table 1.

<sup>2)</sup>Values with different superscript letters within a column are significantly different from each other group at  $p < 0.05$ .

<sup>3)</sup>Not significant difference between non exercise stress group and 2 hour exercise stress group by *t*-test.

\* $p < 0.05$ .

### Changes of antioxidant system according to regular exercise and vitamin C supplementation

Experimental animals were categorized into the 2-hour exercise stress group and the non stress group to examine antioxidant enzyme activity, and this is shown in Table 3. The results showed that when any exercise stress was not experienced, there was a significant increase in the activation of GSH-Px for the T group with regular exercise as compared to the control group, while there was no significant change when vitamin C was supplemented. Among the group with exercise stress, the T and TC groups showed significant increases than the C group.

When SOD activities were restricted by converting the superoxide radical to H<sub>2</sub>O<sub>2</sub>, peroxidation started to appear gradually so that the integrity of the cell membrane was damaged, and H<sub>2</sub>O<sub>2</sub> was converted by catalase and GSH-Px to H<sub>2</sub>O (14). There was a significant increase ( $p < 0.05$ ) in serum SOD activities for the TC group as compared to the T group when the mice were non-stressed, while there was no difference between the C and T groups. Those with a 2-hour exercise stress showed SOD activity levels in the following order: C group < T group < TC group, with a significant difference between the three groups ( $p < 0.05$ ). They were known to have more effects of regular exercise and vitamin supplementation than these of the group with no exercise stress.

There was no difference in the MDA concentrations between the experimental groups amongst those not experiencing exercise stress (Table 4). As well, there was no differences between the C and T groups in the 2 hour exercise stress group. However, the TC group showed the MDA concentration significantly lower than that of the T group so that the effects of vitamin C supplementation on exercise stress appeared to be higher than those of regular training.

Recently, the effects of antioxidant supplementation on exercise performance have been increasingly studied. It has been reported that sudden vigorous exercise and long-term training result in a greater consumption of various antioxidants so that it becomes necessary to take in plenty of fruits with  $\beta$ -carotene and vitamin C as an-

**Table 4.** MDA concentration in the experimental group

Groups <sup>1)</sup>	MDA (nmole/mg prot)		<i>t</i> -test
	Non exercise stress	2 hr exercise stress	
C	1.48 ± 0.16	2.82 ± 0.41 <sup>a2)</sup>	*
T	1.78 ± 0.18	2.55 ± 0.20 <sup>a</sup>	NS <sup>3)</sup>
TC	1.52 ± 0.17	1.32 ± 0.22 <sup>b</sup>	NS

<sup>1)</sup>Groups are the same as Table 1.

<sup>2)</sup>Values with different superscript letters within a column are significantly different from each other group at  $p < 0.05$ .

<sup>3)</sup>Not significant difference between non exercise stress group and 2 hour exercise stress group by *t*-test.

\* $p < 0.05$ .

tiioxidant (15). In addition, antioxidant supplementation has been reported to improve exercise performance and reduce the oxidative stress-following exercise so that muscular damage can be reduced (16). In fact, Hyun (17) reported that there was a significant increase in SOD and catalase activities immediately following exercise and during recovery time (15 minutes), due to the long-term vitamin C and E supplementation, compared to those of a control group. Sastre et al. (18) further reported that vitamin C contributed to preventing the serum GSH from being oxidized during exercise. By contrast, Choi et al. (9) reported that the supplementation of multiple vitamin oxidant containing  $\beta$ -carotene and vitamin C led to a significant increase in MDA concentration after 8 weeks of supplementation, when taken in conjunction with an exercise intensity level of 85% maximum heart rate.

### Changes of antioxidant system according to exercise stress

In this study, there was no difference in the GSH-Px activities between the 2-hour exercise stress group and the non-exercise stress group. Based on the control group, it was shown that the 2-hour exercise stress group had a significant reduction in the SOD activities, and both the T and TC groups were not influenced by exercise stress (Table 3). As a result of several studies, the effects of exercise on the antioxidant system have been reported to be contrary. Buczynski et al. (19) reported, for example, that there was an increase in antioxidant enzymes

such as serum SOD and catalase for 20 minutes after exercise on 75% VO<sub>2</sub>max, while Kim et al. (20) reported that serum SOD and catalase were significantly increased by exhaustive exercise following the long-term endurance training with rats, and Ohno et al. (21) indicated that any significant change was not actually caused by the serum antioxidant enzyme after exercise of the same exercise intensity level.

A significant increase in MDA concentration was observed in the C group when exercise stress was provided, yet without any significant difference in the T and TC groups. As a result, regular exercise and vitamin C supplementation can be considered important in controlling the formation of the lipid peroxides caused by exercise stress (Table 4). This means that vitamin C supplementation improves the antioxidant system of the body so that the damage to cell tissues caused by oxidative stress can be reduced (22). Studies on exercise and lipid peroxide showed that there was a greater increase in oxygen radicals during exercise than during periods of non-exercise, as well as an increase in lipid peroxide in proportion to exercise intensity (23). As well, it was shown that lipid peroxide increased after exercise conducted with a 100% maximum oxygen intake (24).

This study has demonstrated that the antioxidant enzyme was increased by proper and regular training with the exercise stress group, while SOD activity was increased by vitamin C supplementation. Moreover, a decrease in MDA concentration was observed in the 2-hour exercise stress group by vitamin C supplementation. It seems, therefore, that vitamin C supplementation during exhaustive exercise can maximize its action as an antioxidant, thus helping enhance exercise performance.

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