

## **Green Tea Maintains Antioxidative Defense Enzyme Activities and Protects Against Lipid Peroxidation in Rat Gastrocnemius Muscles After Aerobic Exercise**

Young-Mi Chai and Soon-Jae Rhee<sup>†</sup>

*Department of Food Science and Nutrition, Catholic University of Daegu, Gyeongsan 712-702, Korea*

### **Abstract**

This study investigated the effects of green tea on the muscle antioxidative defense system in the white & red gastrocnemius muscles of rats after aerobic exercise. Male Sprague-Dawley rats weighing  $150 \pm 10$  g were randomly assigned to a control group, non-exercise with green tea group (G group), and exercise training group. The exercise training group was then further classified as the training (T) group and training with green tea (TG) group, the latter of which was supplemented with green tea in the drinking water during the experimental period. The rats in the exercise training groups (T and TG) were subjected to aerobic exercise on a treadmill 30 min/day at a speed of 28 m/min (7% incline) 5 days/week, while the other groups (control and G group) were cage confined for 4 weeks. Thereafter, the rats were sacrificed with an injected overdose of pentobarbital just after running. In the white muscle, the xanthine oxidase (XOD) activities were 71% higher in the T group compared to control group, whereas the TG group had the same activity as the control group. The XOD activities in the red gastrocnemius muscle exhibited the same tendency as in the white muscle. The superoxide dismutase (SOD) activity in the white muscle was lower in the T group compared with the control group, yet significantly higher in the TG group compared with the T group. The SOD activities in the red gastrocnemius muscle exhibited the same tendency as in the white gastrocnemius muscle. The glutathione peroxidase (GSHpx) activities in the white & red gastrocnemius muscles were 43% lower in the T group compared with the control group, yet the activities in the TG group remained at control levels. The glutathione S-transferase (GST) activity in the white muscle was not significantly different among any of the three groups, but in the red gastrocnemius muscle, the TG group had the same activity as in the control group. The thiobarbituric acid reactive substance (TBARS) contents in the white & red gastrocnemius muscles were higher in the T group than in the control but the control and TG groups had the same concentrations of TBARS. In conclusion, the supplementation of green tea in rats subjected to aerobic exercise was found to reduce the peroxidation of muscle lipids by enhancing the antioxidative defense mechanism.

**Key words:** green tea, aerobic exercise, antioxidative defense system, white gastrocnemius, red gastrocnemius

### **INTRODUCTION**

With the recent rapid economic growth, the resulting changes in living environments and westernized dietary habits have led to an increase in circulatory diseases, such as hypertension, arteriosclerosis, heart attacks, and brain diseases, which are now the leading causes of death in Korea (1,2). As a result, there has been a slow increase in the number of people attempting to improve their dietary habits and using aerobic exercise to alleviate the threat of circulatory diseases. Aerobic exercise is designed to overload the heart and lungs, causing them to work harder than at rest. The increased activity increase both glucose and lipid uptake and oxidation by muscle and improves the lipid composition of the blood, with

health benefits that include curtailing the effects of metabolic syndrome, improving the regulation of the immune and endocrine systems, and reducing the changes of heart attack.

In spite of the above advantages of aerobic exercise, it is also a concern that excessive exercise can lead to serious health problems, such as diseases and infections. In addition, it has been reported that excessive-exercise causes an increase in radical and oxidative stress due to the leakage of electrons during the increased activity of the electron transfer system (3). When a large amount of reactive oxygen is accumulated and exceeded the protective capacity of the cellular antioxidative mechanism, the cell membrane's unsaturated fatty acids can be oxidized. Moreover, it has been reported that the reactive

<sup>†</sup>Corresponding author. E-mail: sjrhee@cu.ac.kr  
Phone: +82-53-850-3523, Fax: +82-53-850-3504

oxygen resulting from lipid peroxidation is so excessive that enzymes and other substances become inactive, resulting in serious damage to the muscular system (4).

The internal antioxidative mechanism that protects against reactive oxygen includes antioxidative defense enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHpx), plus non-enzymatic substances, like vitamin E and C,  $\beta$ -carotene, uric acid, bilirubin, albumin, and glutathione, which can reduce reactive oxygen into a more stable substance. As such, the reactions involved in the antioxidative system are very important for protecting the human body from oxidative damage. Previous studies have already shown that antioxidants like vitamin E and C, selenium and catechin play a significant role in activating the antioxidative system (5,6).

In a study of the relationships between physical exercise and antioxidants, Gohil et al. (7) reported that an insufficiency of antioxidative substances results in an increase in the lipid peroxidation of skeletal muscles, as well as a decrease in endurance. It has also been reported that damage to the skeletal muscles and the oxidation of protein can be reduced if vitamin E, a powerful antioxidant, is supplied before and after long-sustained exercise (8).

Accordingly, many recent studies have focused on the use of antioxidants to reduce oxidative stress resulting from excessive exercise (9-11). For example, Choi et al. (12) demonstrated that glucuronic acid helps to prevent oxidative damage in the liver and white gastrocnemius muscles fibers in rats during recovery from muscular fatigue after aerobic exercise. In addition, Atalay et al. (13) reported that vitamin E helps the liver and red gastrocnemius muscle fibers enhance their antioxidative ability in rats subjected to aerobic exercise. Meanwhile, Khanna et al. (14) found that feeding lipoic acid (LA) to rats subjected to aerobic exercise improves the antioxidative reaction of the red gastrocnemius muscle fibers, and also reduces lipid peroxidation. Finally, special attention has been paid to other antioxidants, including vitamin B<sub>6</sub>, vitamin C, and glucuronic acid, which are also believed to reduce the oxidative stress and free radical generation resulting from physical exercise (15,16).

Various medicinal effects of green tea, have already been reported (17-19). Furthermore, it is already known that catechin, a functional substance found in green tea, is particularly powerful antioxidant, and very effective at scavenging endogenous free radicals generated by stress in the human body (20,21). In a previous related study by Kim and Rhee (22), green tea was found to help prevent oxidative damage in the liver of rats re-

covering from muscle fatigue after aerobic exercise.

Accordingly, in the current study, rats were subjected to aerobic exercise on a treadmill, then the effect of green tea on antioxidative detoxification was observed in the major active muscles, the red and white gastrocnemius.

## MATERIALS AND METHODS

### Preparation of experimental animals, diet and green tea beverage

Male Sprague-Dawley rats weighing  $150 \pm 10$  g were purchased from KRITC (Korea Research Institute of Chemical Technology, Daejeon, Korea). The animals were housed individually in stainless steel cages in a room with controlled temperature ( $20 \sim 23^\circ\text{C}$ ) and lighting (alternating 12 h periods of light and dark) and fed a pelletized, commercial non-purified diet for 6 days after arrival. Next, the rats were randomly divided into a control group, non-exercise with green tea group (G group), and exercise training group. The exercise training group was divided into sub-groups with (TG) and without (T) green tea. The green tea was added as a supplement to the drinking water during the experimental period (Table 1). The experimental animals were all fed a commercial non-purified diet (Samyang, Seoul). The green tea beverage used for the experiment was prepared by steeping 5 g of green tea leaves in 100 mL of distilled water at  $85^\circ\text{C}$  for 3 minutes, based on the use of commercially available tea bags manufactured by Taepyungyang Co., Ltd.

### Exercise training

A motor-driven treadmill (Junkok Inc, Korea) was used to subject the rats to exercise under the conditions shown in Table 2. The rats ran five times a week on the treadmill set at a 7% slope and a speed of 10 m/min for the first week, 20 m/min for the second week, 25 m/min for the third week and 30 m/min for the fourth week, respectively.

**Table 1.** Classification of experimental groups

Groups	Treadmill	Drinking water
Control <sup>1)</sup>	-	Distilled H <sub>2</sub> O
G <sup>2)</sup>	-	Green tea <sup>5)</sup>
T <sup>3)</sup>	+	Distilled H <sub>2</sub> O
TG <sup>4)</sup>	+	Green tea <sup>5)</sup>

<sup>1)</sup>Control: basal diet + distilled H<sub>2</sub>O.

<sup>2)</sup>G: basal diet + green tea.

<sup>3)</sup>T: basal diet + training + distilled H<sub>2</sub>O.

<sup>4)</sup>TG: basal diet + training + green tea.

<sup>5)</sup>5% tea extract solution: 5 grams of dry tea leaves were added to 100 mL of hot distilled water in a beaker and extracted at  $85^\circ\text{C}$  for 3 min.

**Table 2.** Exercise training schedule for exercised rats

	Duration (week)			
	1	2	3	4
Speed (m/min)	10	20	25	28
Grade (degree)	7	7	7	7
Time (min)	10	20	25	30
Frequency (days/week)	5	5	5	5

### Measurement of enzyme activity

**Preparation of rat gastrocnemius muscle:** After exercising, the rats were anesthetized with pentobarbital (Greencross Co, Korea), following a 12 h-fast. The red gastrocnemius and white gastrocnemius were then removed from the femoral region and stored at  $-70^{\circ}\text{C}$  until analyzed.

**Measurement of xanthine oxidase (XOD) activity:** The XOD activity in the rat gastrocnemius muscle was determined according to the method of Stripe and Della-Corte (23).

**Measurement of antioxidative defense enzyme activity:**

The superoxide dismutase (SOD) activity in the red gastrocnemius was determined according to the method of Marklund and Marklund (24). The glutathione peroxidase (GSHpx) activity in the red gastrocnemius was determined according to the method of Lawrence and Burk (25). The glutathione-S-transferase (GST) activity in the red gastrocnemius was determined according to the method of Habig et al. (26).

**Determination of lipid peroxide in red gastrocnemius:**

The lipid peroxide in the rat gastrocnemius muscle was determined by the Satoh's method (27), which quantifies substance reacting to thiobarbituric acid (TBA).

**Protein determination:** The protein in the rat gastrocnemius muscle was determined according to the method of Lowry et al. (28) using bovine serum albumin as the standard.

### Statistical analysis

The results were assessed by ANOVA and Tukey's honestly significant difference test. Differences were considered significant at  $p < 0.05$  (29).

## RESULTS

### Green tea intake

Green tea intakes were 20.75 and 25.21 mL/day in the G and TG groups, respectively. The green tea intake of the TG group was higher than the G group (Table 3).

### Body weight gain

Body weight gain was lower in exercise training group, compared to control group, but it was significantly different between exercise training groups (Table 4).

**Table 3.** Water and green tea intake in rats

Group	Water intake (mL/day)	Green tea intake (mL/day)
Control	$21.5 \pm 1.85$	-
G	-	$20.75 \pm 2.65$
T	$23.0 \pm 2.15$	-
TG	-	$25.21 \pm 2.50$

**Table 4.** Effect of green tea on body weight gain

Group	Body weight gain (g)
Control	$252.5 \pm 5.85^a$
G	$253.1 \pm 4.67^a$
T	$215.8 \pm 7.50^b$
TG	$218.2 \pm 8.90^b$

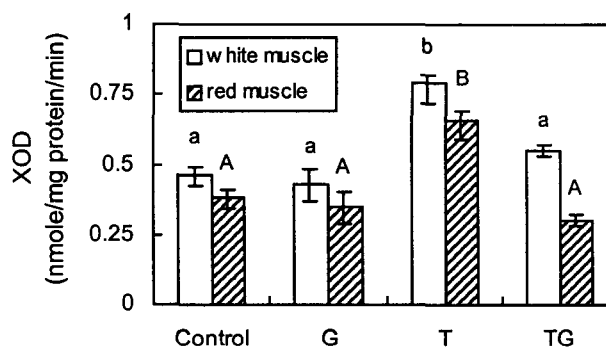
### Xanthine oxidase (XOD) activity in gastrocnemius muscles

In the white gastrocnemius muscle, the activity of XOD, known as a free radical generation enzyme, increased by 71% in the T group compared to the control group, whereas the TG group had the same activity level as in the control group. The XOD activity in the red gastrocnemius muscle exhibited the same tendency as in the white gastrocnemius (Fig. 1).

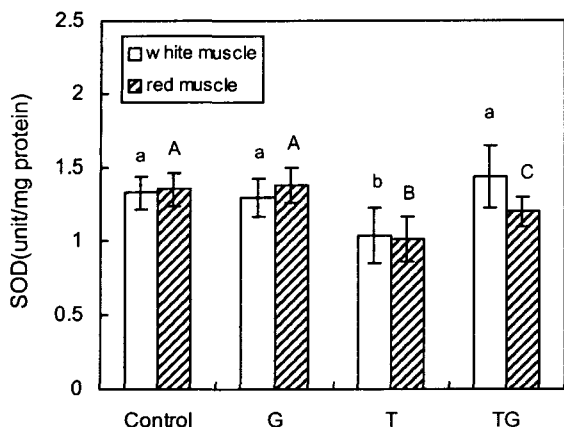
### Superoxide dismutase (SOD) activity in gastrocnemius muscles

SOD is an enzyme that protects the body from oxygen toxicity by converting superoxide radicals into  $\text{H}_2\text{O}_2$ . The activity of SOD in the gastrocnemius muscles is presented in Fig. 2.

In the white gastrocnemius muscle, the SOD activity was significantly lower in the T group compared with the control group, whereas the TG group, with green tea supplementation, had the same SOD activity as the control group. In the red gastrocnemius muscle, the SOD activity in the T group was 20% lower than the control group, however, SOD activity in the TG group, with



**Fig. 1.** Effect of green tea on gastrocnemius muscles xanthine oxidase (XOD) activities after aerobic exercise in rats. All values are mean  $\pm$  SE ( $n=10$ ). Bars with different letters are significantly different at  $p < 0.05$  based on Tukey's-HSD test. The experimental conditions were the same as in Tables 1 and 2.



**Fig. 2.** Effect of green tea on gastrocnemius muscles superoxide dismutase (SOD) activities after aerobic exercise in rats. All values are mean  $\pm$  SE (n=10). Bars with different letters are significantly different at  $p < 0.05$  based on Tukey's-HSD test. The experimental conditions were the same as in Tables 1 and 2.

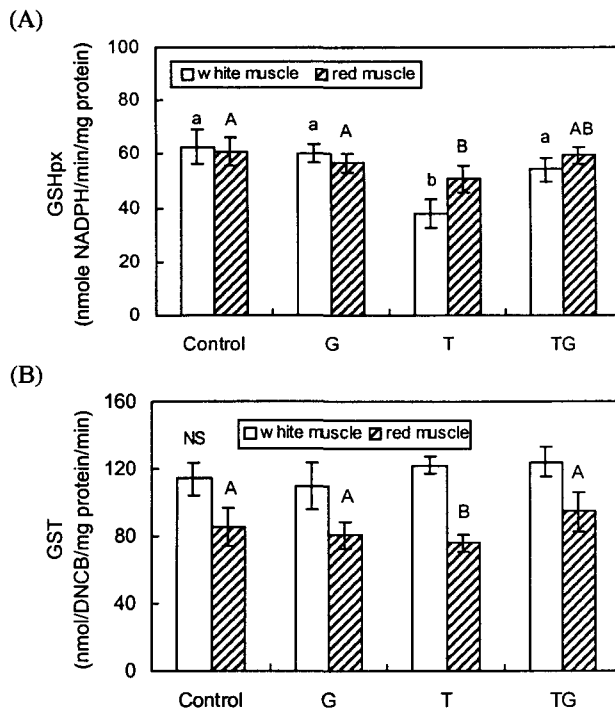
green tea supplementation, was again significantly higher than the T group.

#### Glutathione peroxidase (GSHpx) and glutathione S-transferase (GST) activity activity in gastrocnemius muscles

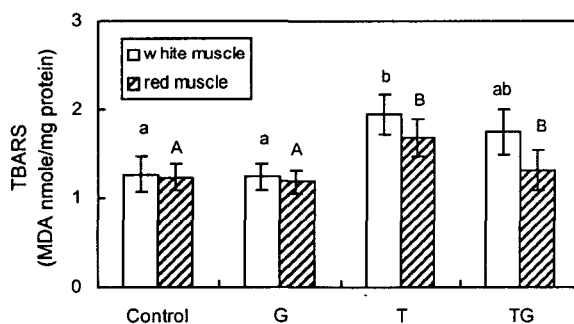
GSHpx catalyzes the formation of oxidized glutathione (GSSG) and  $H_2O$  from  $H_2O_2$  and reduced glutathione (GSH), along with the formation of alcohol and  $H_2O$  from peroxides (ROOH). In the white gastrocnemius, the GSHpx activity was 43% lower in the T group compared to the control group, whereas the TG group retained the same level as in the control group due to the green tea supplementation. In the red gastrocnemius muscle, the GSHpx activity was 17% lower in the T group compared to the control group, whereas the TG group exhibited the same activity as in the control group. In the white gastrocnemius muscle, the GST activity in the exercise training group was not different from that in the control group. However, in the red gastrocnemius muscle, the GST activity was 11.3% lower in the T group compared to the control group, whereas the TG group retained the same level as in the control group due to the green tea supplementation (Fig. 3).

#### Lipid peroxide levels in gastrocnemius muscles

The lipid peroxide concentrations, as an index of lipid peroxidation are presented in Fig. 4. In the white gastrocnemius muscle, the TBARS concentrations as an index of lipid peroxidation were 31% higher in the T group compared with the control group. However, in the TG group with green tea supplementation, the TBARS concentrations remained at the same level as in the control group. In the red gastrocnemius muscle, the TBARS



**Fig. 3.** Effect of green tea on gastrocnemius muscles glutathione peroxidase (GSHpx, A) and glutathione S-transferase (GST, B) activities after aerobic exercise in rats. All values are mean  $\pm$  SE (n=10). Bars with different letters are significantly different at  $p < 0.05$  based on Tukey's-HSD test. The experimental conditions were the same as in Tables 1 and 2.



**Fig. 4.** Effect of green tea on gastrocnemius muscles thiobarbituric acid reactive substances (TBARS) after aerobic exercise in rats. All values are mean  $\pm$  SE (n=10). Bars with different letters are significantly different at  $p < 0.05$  based on Tukey's-HSD test. The experimental conditions were the same as in Tables 1 and 2.

concentrations were significantly higher in the T group compared to the control group. However, in the TG group with green tea supplementation, the TBARS concentrations were same as in the control group.

## DISCUSSION

This study evaluated the efficacy of green tea for protecting exercising skeletal muscle from oxidative damage. Rats were subjected to aerobic exercise using a

treadmill, and then changes in the free radical generation system and free radical scavenging system in the gastrocnemius muscles were examined, along with related oxidative damage.

XOD, one of the free radical generation systems in the human body, is a non-specific enzyme that is related to the metabolism of purines, pyrimidines, aldehydes, and heterocyclic compounds. Hypoxanthine, a metabolic product resulting mainly from purine metabolism, is oxidized into xanthine, which is then re-oxidized and acts as a reactive catalyst generating uric acid, plus free radicals (30). In this study we found that the XOD activity in both the white and red gastrocnemius muscle fibers was remarkably higher in the T group than in the control group, while the green tea-supplemented TG group exhibited a significantly lower activity that remained at the same level as in the control group. This result is similar to the report by Laughlin et al. (31), where XOD activity increased in the skeletal muscle after physical exercise, and the report by Kim and Rhee (10), where XOD activity increased in the gastrocnemius after aerobic exercise using a treadmill, yet decreased with the supply of glucuronic acid.

SOD reduces superoxide radicals into  $H_2O_2$ , which is then made non-toxic by on the action of GSHpx and catalase, thereby protecting the human body from oxygen poisoning (32). In this study, the T group exhibited a significant reduction in SOD activity in both the white and red gastrocnemius muscle when compared with the control group, whereas the TG group with green tea supplementation, retained almost the same activity as in the control group. This result is also in agreement with the reports by Choi et al. (12) and Kim and Rhee (10), where reduced SOD activity in the white gastrocnemius after aerobic exercise using a treadmill was maintained when glucuronic acid was supplied, and the report by Oh (33), where the addition of antioxidants during sustained exercise produced a significant increase in SOD activity.

GSHpx catalyses the generation of  $H_2O$  and oxidized glutathione (GSSG) from  $H_2O_2$  and reduced glutathione (GSH), and the generation of alcohol (ROH) and  $H_2O$  from peroxide (ROOH) (34). In this study, the T group exhibited lower GSHpx activity in the white and red gastrocnemius than the control group, while the TG group showed an equivalent level to the control group. This result is similar to the report by Choi et al. (12) and Kim and Rhee (10), where GSHpx activity increased in the liver and white gastrocnemius muscle when glucuronic acid was supplied after aerobic exercise.

GST is a major Phase II detoxification enzyme that catalyzes reactions that conjugate glutathione (R-S-G) to

numerous electrophilic substances including carcinogen and endogenous poison (26). Although the current experiment did not reveal any significant difference in the GST activity in the white gastrocnemius muscle, in red gastrocnemius muscle the T group exhibited a 23% decrease in GST activity when compared with the control group. However, green tea supplementation maintained the GST activity at the same level as the control group. This result is similar to the report by Khanna (14), where the antioxidative activity, including the GST activity, in the red gastrocnemius was increased by providing lipoic acid (LA) after aerobic exercise.

As mentioned above, green tea supplementation prevented the decrease in SOD, GSHpx, and GST activities in response to aerobic exercise that was seen in the exercised group without green tea, which indicates that the catechin, vitamin E, and vitamin C contained in green tea function as antioxidants, thereby protecting the cell membranes of sub-organs from peroxidation.

Lipid peroxidation is accelerated by the decrease in antioxidative substances and the increase in free radical generation caused by oxidative stress within cells (35). The lipid peroxides contents (TBARS concentrations), an indicator of oxidative damage, were significantly higher in both gastrocnemius muscles in the T group when compared to the control group. This finding is similar to the report by Sen et al. (36), where the lipid peroxidation caused by exercise was partly restricted by supplying vitamin E during exercise.

Accordingly, the current study found that the supplementation of green tea produced protective effects against oxidation and oxidative damage in the gastrocnemius muscles in rats after aerobic exercise using a treadmill. Furthermore it would seem that these protective effects result from the antioxidative substances in green tea, such as catechin.

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