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Stimulatory Effects of Ferulic Acid on Endurance Exercise Capacity in Mice

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Ferulic acid was orally administered to mice in order to investigate its effects on exercise endurance capacity. When a single administration of ferulic acid was given to the mice in an adjustable-current water pool, the duration of exhaustive swimming was longer than that exhibited by the mice in the control group. Also, when the mice were exhaustively exercised for 3 consecutive days, no change in swimming time was found in the ferulic acid-administered group on the final day, and a large decrease in the untreated mice. Administration of ferulic acid efficiently activated the hepatic antioxidative defense system during exercise. The mice that received ferulic acid showed significant increases in the activity of hepatic antioxidant enzymes such as superoxide dismutase, catalase, and glutathione-*S*-transferase. Furthermore, an increased glutathione level was observed, while the malondialdehyde content was reduced. These results suggest that ferulic acid possesses stimulatory effects that can enhance exercise endurance capacity and reduce fatigue by elevating antioxidative potentials.

Key words: ferulic acid; exercise endurance capacity; fatigue; antioxidation

Exercise is known to promote good health and prevent various diseases. However, strenuous exercise can cause oxidative stress, which leads to an imbalance between reactive oxygen species (ROS) production and antioxidant defense. This imbalance eventually damages biological molecules and key cellular components and processes, such as lipid peroxidation, enzyme inactivation, and oxidative DNA damage.[1,2] ROS accumulation has also been implicated in the aging process.[3]

Appropriate nutritional supplements are widely used for better exercise performance. Several natural components have been found to exert physiological effects, and some of them are considered to be useful for improving athletic performance or avoiding the disturbance of homeostasis caused by strenuous exercise.[4] Supplementation with compounds such as capsaicin, stearoyl vanillylamide, and capsiate has been reported to increase endurance exercise capacity in mice.[5–7] In addition, the prolonged effects of exercising to exhaustion have been observed using phenolic compounds in chronic swimming.[8] Some medical plants and catechin-rich green teas have been found to enhance the capacity for exhaustive exercise in a mouse model by an increase in energy production *via* lipid metabolism.[9,10] Antioxidants might help to reduce the degree of exhaustion caused by continuous physical activity in terms of protection against exercise-induced oxidative stress.[11]

Ferulic acid, 4-hydroxy-3-methoxycinnamic acid, is one of the most ubiquitous phenolic acids, found in the bran of grasses such as wheat, rice, and oats. It belongs to the family of plant hydroxycinnamic acids, which include caffeic acid, sinapic acid, and *p*-coumaric acid. Ferulic acid is as a food preservative because of its antiautoxidative effects on oils,[12] and as an active ingredient in cosmetic products because of its photoprotective effects.[13] Recent studies have provided evidence that ferulic acid reduces the risk of disease, including Alzheimer's disease, cardiovascular disease, diabetes, and colon cancer.[14]

Currently, ferulic acid is used to enhance athletic performance, both in humans and racehorses. Supplementation by it has been found to increase muscle strength in weight lifters.[15] Even though the health benefits of ferulic acid have gained much attention, its influence against exhaustive exercise capacity has not been entirely proven.

In the present study, the stimulatory effects of ferulic acid on exercise endurance and anti-fatigue were investigated *in vivo* using an adjustable-current water pool. Furthermore, its mechanism of action relevant to its antioxidative activity was determined.

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Abbreviations: BHT, butylated hydroxytoluene; CAT, catalase; DPPH, 1,1-diphenyl-2-picrylhydrazyl; GST, glutathione-*S*-transferase; ROS, reactive oxygen species; GSH, reduced glutathione; SOD, superoxide dismutase

Materials and Methods

Chemicals. Ferulic acid was purchased from Sigma-Aldrich Chemie (Steinheim, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), hypoxanthine (XA), xanthine oxidase (XOD), tetrazolium blue, 1,1,3,3-tetramethoxypropane (malondialdehyde; MDA), butylated hydroxytoluene (BHT), l-ascorbic acid, hydrogen peroxide (H_2O_2), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione-*S*-transferase (GST), and Tween 80 were obtained from Sigma Chemical (St. Louis, MO). All other chemicals were of analytical reagent-grade.

Animals. Male ICR mice (4 weeks, 16 ± 2 g of body weight, b.w.) were purchased from Orient Bio (Seongnam, Korea) and housed in cages under automatically controlled airconditions of temperature ($22 \pm 2\,^{\circ}C$), humidity (about 60%), and lighting (12:12-h light-dark cycle). The mice were fed commercial pelleted chow (AIN-76A rodent purified diet, Orient Bio) and water *ad libitum*. Our Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the "Guidelines for Animal Experiments" established by Chonnam National University.

Experimental groups. After 1 week of acclimation, the mice were forced to swim twice at 3 d-intervals for the measurement of exhaustive swimming time. Then, they were grouped for each test with similar mean swimming capacities.

Experiment 1: For the test of the acute effect of ferulic acid on endurance exercise capacity, 18 mice were divided into three groups ($n = 6$ per group) with similar mean swimming capacities, determined in an adjustable-current water pool at a flow rate of 7 l/min: exercised control group (10% tween 80) and two sample-treated groups (125- or 250-μmole/kg b.w. of ferulic acid).

Experiment 2: To evaluate the acute effect of ferulic acid on hepatic antioxidation and lipid peroxidation markers, the animals were divided into three groups of six mice with similar mean swimming capacities: a non-exercised control group (10% tween 80), an exercised control group, and a sample-treated group (250 μmole/kg b.w. of ferulic acid). The swimming protocol was same as in experiment 1.

Experiment 3: For the anti-fatigue test, the mice were separated into two groups (control and sample treated groups; $n = 10$ per group) with similar mean swimming capacities, determined in an adjustable-current water pool at a flow rate of 8 l/min.

DPPH radical scavenging activity. The free radical scavenging activity of the tested sample was measured by DPPH assay, with some modifications.[16] Briefly, 0.95 ml of 1.0×10^{-4} M DPPH radical solution in ethanol was prepared, and then mixed with 0.05 ml of the sample dissolved in ethanol. The amount of DPPH remaining in the mixed solution was measured at 515 nm for 30 min. The reduction in absorbance of the DPPH solution indicated the degree to which radical scavenging activity increased. Ethanol without the sample was employed as a control. DPPH radical scavenging activity was calculated according to the following formula: $(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$. The SC_{50} value was defined as the concentration (μM) of sample required for 50% scavenging of the DPPH radical.

NBT reduction assay. The superoxide anion radical ($O_2^{\bullet -}$) scavenging activity of the tested sample was determined by nitroblue tetrazolium (NBT) assay, as described by Nishikimi,[17] with some modifications. A 20-μl sample was added to 980 μl of 0.5 mM XA/NBT mixture and dissolved in 50 mM potassium phosphate buffer (pH 7.4) containing 0.05 mM EDTA. After the XOD (0.05 units) was put into a test tube, the mixture was incubated for 20 min at 37 °C. The reaction was terminated by the addition of 0.5 ml of 2 N HCl, and the absorbance of NBT was measured at 560 nm. The scavenging activity was calculated as follows: Scavenging activity (%) $= [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$. The SC_{50} value was defined as the concentration (μM) of sample required for 50% scavenging of $O_2^{\bullet -}$.

Measurement of exercise endurance capacity. An acryl plastic pool ($90 \times 45 \times 45$ cm) was used to determine swimming capacity.[18] The pool was filled with water to 38 cm and the temperature was maintained at $34 \pm 1\,^{\circ}C$. The current strength was adjusted to 7 l/min by controlling the voltage in the pool pump, and monitored using a water flow meter. Prior to the experiment, the animals were fasted overnight, with water provided *ad libitum*. The mice, which had received the appropriate vehicle or sample by gastric intubation 30 min before swimming without any load were assessed to be exhausted when they failed to rise to the surface of the water to breathe. The index of endurance swimming capacity was measured according to the total swimming period until exhaustion. After the end of the experiment, the mice were sacrificed to collect the liver. This was stored at $-70\,^{\circ}C$.

Determination of anti-fatigue effect. On day 1, all the mice were allowed to swim exhaustively at a flow rate of 8 l/min in the pool to measure the swimming period, and they were divided into control and sample treated groups with similar mean swimming capacities (grouping day). On days 2 and 3, the mice were forced to swim 30 min after vehicle or sample treatment by gastric intubation, and the exhaustive swimming capacity was determined. The difference between the maximum swimming times on the 1st and 3rd trials was measured in order to evaluate the anti-fatigue effect on consecutive swimming exercise. At 12 h before the experiment, the animals were fasted, with water provided *ad libitum*. After the end of the experiment, the mice were sacrificed to collect the liver. This was stored at $-70\,^{\circ}C$.

Assays for hepatic antioxidant activities. For the antioxidant activity assays, the liver was homogenated in 50 mM phosphate buffer. The suspension was then centrifuged at $13{,}000 \times g$ for 15 min at 4 °C, and the supernatant was used for the measurement. SOD activity was measured using an adaptation of the method described by McCord and Fridovich.[19] The activity of CAT was determined as described by Aebi.[20] Hepatic GST activity was assayed by the method of Habig and Jakoby.[21] The level of GSH, a key intracellular antioxidant, was measured by the method of Akerboom and Sies.[22] The concentration of MDA, the end product of lipid peroxidation, was assayed by monitoring thiobarbituric acid reactive substance formation, as described by Draper and Hadley.[23] The amount of protein was measured using the Bradford assay.[24]

Statistical analysis. Data are presented as mean of ±S.E. of three replicates. The data were statistically evaluated using Student's *t*-test or one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test to compare significant differences between the groups at $p < 0.05$.

Results

Effects of ferulic acid on exercise endurance capacity

Exercise endurance capacity was evaluated by measuring the total swimming time until exhaustion of the mice in an adjustable-current water pool. As shown in Fig. 1, the mean duration of exhaustive swimming of the mice treated with a single administration of ferulic acid was significantly longer than that of the control mice. The mice treated with 125 or 250 μmole/kg b.w. of ferulic acid showed approximately 1.4- and 1.7-fold increases in swimming time as compared to the control mice (22.0 ± 1.7 min).

Effects of ferulic acid on hepatic antioxidant enzymes

As presented in Fig. 2, the CAT activity in the exercised control group, which showed an average exhaustive swimming time of 27.5 min (data not shown), decreased by approximately 10% as compared to the non-exercised group. However, a single administration of ferulic acid, which extended the average swimming time to 41.7 min (data not shown), resulted in the prevention of a decrease in CAT activity. Compared to the non-exercised control group, the hepatic SOD

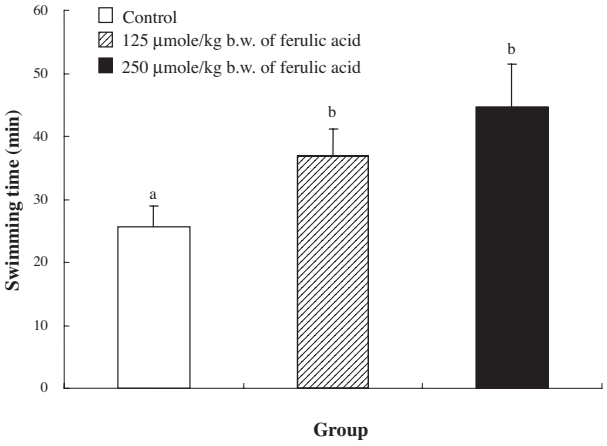

Fig. 1. Effects of Ferulic Acid on Exhaustive Swimming Capacity in Mice.

Data express the mean ± S.E. for 6 mice in each group. Different letters above the bar in the groups indicate statistically significant differences by Duncan's multiple range test ($p < 0.05$).

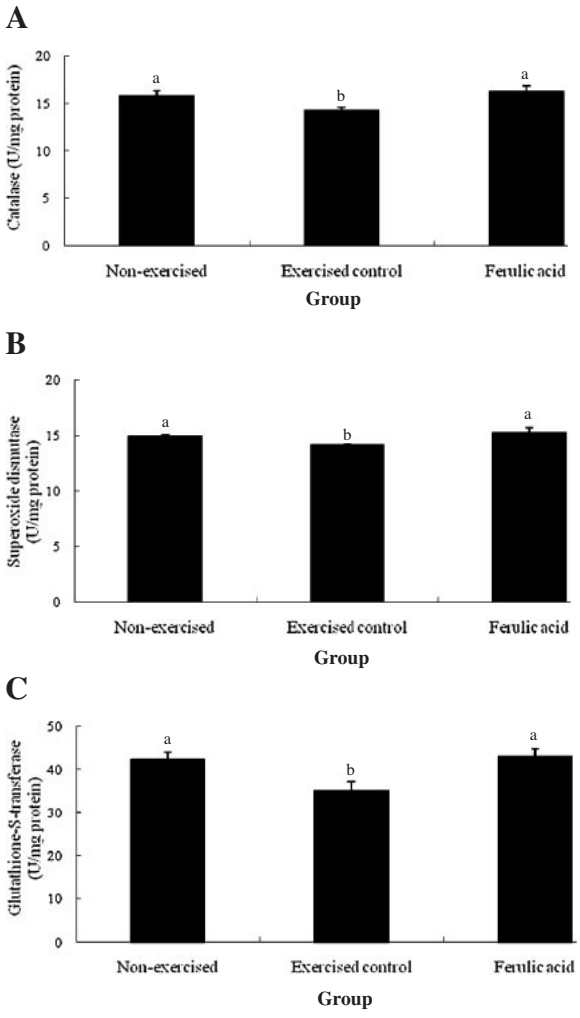

Fig. 2. Effects of Ferulic Acid on Hepatic Antioxidant Enzymes Due to Exhaustive Swimming Exercise in Mice.

The mice were given either vehicle (control) or 250 μmole/kg b.w. of ferulic acid before exhaustive exercise. Data express the mean ± S.E. for 6 mice. Different letters above the bar indicate statistically significant differences by Duncan's multiple range test ($p < 0.05$).

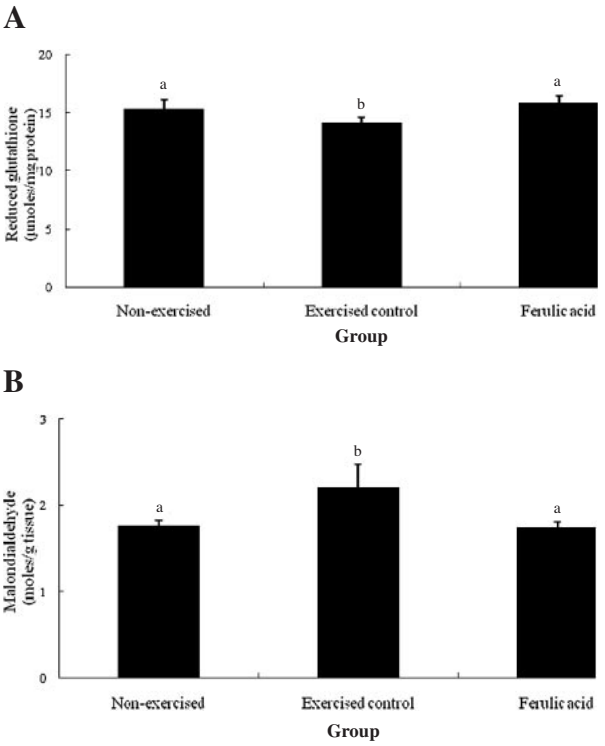

Fig. 3. Effects of Ferulic Acid on Levels of Hepatic Glutathione and Malondialdehyde Due to Exhaustive Swimming Exercise in Mice.

The mice were given either vehicle (non-exercised and exercised control) or 250 μmole/kg b.w. of ferulic acid before exhaustive exercise. Data express the mean ± S.E. for 6 mice. Different letters above the bar indicate statistically significant differences by Duncan's multiple range test ($p < 0.05$).

activity in the exercised control group was also significantly reduced, while no statistical change in SOD activity was found in the ferulic acid-treated mice. Consistently with CAT and SOD activities, pretreatment with ferulic acid significantly protected against the depletion of GST activity induced by exhaustive exercise. On the other hand, a relatively large decrease in GST activity was observed in the control group.

Effects of ferulic acid on GSH and MDA levels

The results of hepatic GSH and lipid peroxidation assays are summarized in Fig. 3. The concentration of hepatic GSH in the exercised control group was lower than in the non-exercised group. However, pretreatment with ferulic acid significantly elevated the hepatic GSH level in comparison with the exercised control group. As shown in Fig. 3, the concentration of MDA, an end product of lipid peroxidation, in the exercised mice that did not receive ferulic acid increased by 1.3-fold as compared to the non-exercised mice. On the other hand, MDA accumulation in the ferulic acid-pretreated mice was similar to that observed in the non-exercised mice.

Anti-fatigue effects of ferulic acid

The changes in exhaustive swimming capacities during the 3-d period of consecutive exercise are presented in Fig. 4. On the initial day (grouping day), there was no significant difference in the exhaustive swimming time between the control and ferulic acid-administered groups (20.4 min *vs.* 20.2 min respectively). Also, similar initial body weights were found in both

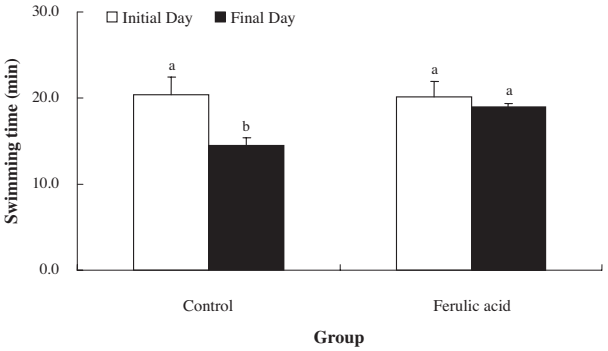

Fig. 4. Anti-Fatigue Effects of Ferulic Acid on Consecutive Swimming Exercise in Mice.

The mice were given either vehicle (control) or 250 μmole/kg b.w. of ferulic acid before exhaustive exercise. Data express the mean ± S.E. for 10 mice in each group. Different letters above the bar in the groups indicate statistically significant differences by Student's *t*-test ($p < 0.05$).

Table 1. Changes in Enzymatic Antioxidant Activities Due to Administration of Ferulic Acid during Consecutive Swimming Exercise[1]

Group	CAT (U/mg protein)	SOD (U/mg protein)	GST (U/mg protein)
Control group	13.53 ± 0.58^{a3}	14.89 ± 0.13^{a}	34.84 ± 0.19^{a}
Ferulic acid-administered group[2]	15.62 ± 0.34^{b}	15.99 ± 0.51^{b}	42.89 ± 1.20^{b}

[1]Data express the mean ± S.E. for 10 mice.
[2]Single oral administration of 250 μmole/kg b.w. of ferulic acid before exhaustive exercise.
[3]Values with different letters in a column are significantly different by Student's *t*-test ($p < 0.05$).

groups (29.7 ± 0.8 g *vs.* 29.4 ± 0.8 g respectively). On the final day, the weight gains of the two groups were not significantly different (final b.w., 31.3 ± 0.6 g *vs.* 30.5 ± 1.2 g respectively). However, the swimming capacity of the control mice drastically decreased, by 29%, while no decrease in swimming time was observed in the mice pretreated with ferulic acid.

Changes in hepatic antioxidant status by fatigue

The antioxidant effects of ferulic acid on the fatigue-induced depletion of antioxidant enzymes are shown in Table 1. The activities of CAT, SOD, and GST in the ferulic acid-administered mice were significantly enhanced in comparison to the control group. Pretreatment with ferulic acid in the mice lead to a lower level of MDA than in the control mice (Table 2). Also, the hepatic concentration of GSH was found to increase by 21% in the ferulic acid group as compared to the control group.

Scavenging effects of ferulic acid on free radicals

The antioxidant potentials of ferulic acid were evaluated according to the scavenging activities on DPPH- and superoxide anion-radicals. Its radical scavenging activities are shown in Table 3. Compared to BHT, a well-known synthetic antioxidant, ferulic acid exhibited relatively high DPPH radical scavenging activity, with an SC_{50} value of 48.31 ± 1.28 μM. However, its activity was slightly less than that of vitamin C, an effective natural antioxidant ($SC_{50} = 33.79 \pm 1.46$ μM).

Table 2. Changes in Hepatic Glutathione (GSH) and Malondialdehyde (MDA) Levels Due to Administration of Ferulic Acid during Consecutive Swimming Exercise[1]

Group	GSH (μmoles/mg protein)	MDA (moles/g tissue)
Control group	12.19 ± 1.02^{a3}	4.90 ± 0.34^{a}
Ferulic acid-administered group[2]	14.77 ± 0.59^{b}	3.88 ± 0.04^{b}

[1]Data express the mean ± S.E. for 10 mice.
[2]Single oral administration of 250 μmole/kg b.w. of ferulic acid before exhaustive exercise.
[3]Values with different letters in a column are significantly different by Student's *t*-test ($p < 0.05$).

Table 3. Scavenging Activities of Ferulic Acid on DPPH- and Superoxide Anion-Radicals[1]

Compound	DPPH radical scavenging activity (SC_{50}[2], μM)	Superoxide radical scavenging activity (SC_{50}, μM)
Ferulic acid	48.31 ± 1.28^{a4}	23.70 ± 1.77^{a}
Vitamin C	33.79 ± 1.46^{b}	23.96 ± 3.35^{a}
BHT[3]	58.55 ± 0.45^{c}	48.18 ± 6.55^{b}

[1]Data express the mean ± S.E. of three replicates.
[2]Concentration required for 50% scavenging of radicals.
[3]Butylated hydroxytoluene.
[4]Values with different letters in a column are significantly different by Duncan's multiple range test ($p < 0.05$).

As shown in Table 3, ferulic acid revealed strong scavenging ability towards $O_2^{\bullet -}$ with 23.70 ± 1.77 μM at SC_{50}, comparable to that of vitamin C. Less scavenging activity on $O_2^{\bullet -}$ was found in BHT.

Discussion

In the present study, the swimming capacity test was chosen over other exercise tests, since it allows for reliable and reproducible evaluation of physical work capacity in mice.[11] The exhaustive swimming time for the ferulic acid-administered mice increased significantly over the control group, implying that ferulic acid might be responsible for the stimulatory effect on endurance exercise capacity. Continuous exercise usually causes fatigue, which can be inhibited by the administration of nutritional regimens, suppressing fatigue relevance factors.[25,26] No decrease in swimming capacity by pretreatment with ferulic acid after consecutive exercise indicates that the prolonged time to fatigue was due mainly to the effect of ferulic acid. Thus, ferulic acid was identified to possess performance-enhancing effects.

Although regular exercise improves well-being, it can be associated with oxidative stress. Thus, exercise can act as a powerful source of ROS, depending on duration and intensity. During exhaustive exercise, fat is typically used as the primary energy source, thus sparing glycogen stores, which in turn retards fatigue.[9] However, substantial production of ROS occurs *via* β-oxidation during the utilization of fat.[27] In addition, a dramatic increase in oxygen consumption in the body takes place as a result of strenuous exercise. In the

presence of oxygen, $O_2^{\bullet -}$ and H_2O_2 are generated in intermediate metabolism. These can lead to an imbalance between ROS and the antioxidant defense system in the body, resulting in the development of fatigue.[28] High levels of oxidative damage due to exhaustive swimming decreased the endurance exercise capacity and increased the fatigue, as evidenced by a significant elevation in the hepatic MDA level and significant decreases in GSH concentration and CAT, SOD, and GST activities, suggesting a role of oxidative stress in exercise performance. Elevation in lipid peroxidation and reduction in intracellular antioxidant activities caused by fatigue have been reported in the mouse model, which is in agreement with the results obtained in the present study.[29]

Exercise-induced oxidative stress is characterized by increased concentrations of hepatic MDA, a major reactive aldehyde resulting from the peroxidation of polyunsaturated fatty acids in the cell membrane.[30] It is important to stabilize lipid peroxidation, since lipid peroxyl radicals initiate the lipid peroxidation chain reaction. In the present study, a significant decrease in the hepatic MDA concentration confirms that a single oral dose of ferulic acid can effectively protect against the hepatic lipid peroxidation induced by exercise stress.

The antioxidant defense system in the body plays an important role in protection against oxidative stress. Inactivation of hepatic antioxidants during exhaustive exercise is caused by exposure to lipid peroxides or ROS. The principal antioxidants against oxidative stress are GSH, CAT, SOD, and GST. GSH is an important non-enzymatic antioxidant that is involved in various enzymatic processes that reduce peroxides and free radicals.[31] In the present study, the level of hepatic GSH remained the same after pretreatment with ferulic acid in exhaustively exercised mice. This indicates that detoxification of ROS, which are responsible for exercise-induced protein oxidation, leads to the prevention of fatigue. Exercise-induced oxidative stress results from exhaustion of endogenous antioxidant defense mechanisms by excess ROS.[32] These can inactivate enzymatic antioxidants. As expected, antioxidant enzymes, including CAT, SOD, and GST, were partially inactivated by intense exercise, which might have been largely due to increased production of ROS. Continuous exercise causes oxidative stress and fatigue, which can be inhibited by the administration of nutritional antioxidants.[29,33] The present study revealed that pretreatment with ferulic acid exhibited a significant protective effect against inactivation of enzymatic antioxidants in mice, and thereby improved exercise capacity and diminished fatigue.

The role of ROS is recognized by increased levels of non-enzymatically induced lipid peroxidation, which in turn influence various enzymatic activities in the body. Thus, it can be linked to enzymatically induced oxidation.[34] Ferulic acid administration decreased lipid peroxidation and improved antioxidant status, due mainly to the antioxidant-sparing action of ferulic acid. Previous studies have shown that phenolic compounds act as antioxidants with increased exercise capacity.[9–11] Ferulic acid is a phenolic compound and an effective scavenger of free radicals. It is also known to decrease lipid peroxidation by inhibiting cytochrome P450, a free radical generator.[35] Our results revealed that the DPPH radical scavenging ability of ferulic acid was high in comparison to that of the synthetic antioxidants. Also, ferulic acid evidenced a comparable degree of $O_2^{\bullet -}$ scavenging activity to natural antioxidants. Based on these results, ferulic acid was implicated as a potentially useful radical scavenger for a host of radicals. These findings suggest that overwhelming ROS due to exhaustive exercise can be scavenged by antioxidant supplementation, which subsequently stabilizes the intracellular antioxidant defense systems. Thus, it might provide the necessary protection against muscle damage and delay exercise-induced muscle fatigue, and thereby enhance exercise performance. This proposed mechanism is supported by a report that vitamin E, an effective free radical scavenger, stabilized membranes and minimized muscle damage during exercise in an animal model, which in turn increased exercise endurance capacity.[36]

In summary, exhaustive exercise enhances lipid peroxidation and ROS production, thereby increasing the risk of inducing irreversible damage due to oxidative stress. However, these adverse effects can be avoided by antioxidant supplementation. Our observations showed that intracellular antioxidant defense systems, including GSH, CAT, SOD, and GST, were stabilized by ferulic acid in the presence of oxidative stressors and that concomitantly, ferulic acid reduced lipid peroxidation. This suggests that improved exhaustive exercise capacity and alleviated fatigue due to ferulic acid in the exhaustive exercise model might be due in part to the protective effects against the oxidative stress induced by exercise, probably *via* the protection of exercised-induced muscle damage. To date, there is insufficient evidence on antioxidants regarding the relationship between the antioxidant defense system and exercise performance. To the best of our knowledge, the present study is the first to investigate the effects of the antioxidant activity of ferulic acid on exercise endurance capacity and anti-fatigue. The results indicate that ferulic acid can be a useful performance-enhancing agent for ameliorating oxidative damage by scavenging radicals and decreasing lipid peroxidation.

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