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EFFECT OF EXERCISE AND α-LIPOIC ACID SUPPLEMENTATION ON OXIDATIVE STRESS IN RATS

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Abstract. We investigated the effect of exercise (running on a treadmill) and α-lipoic acid supplementation for 6 weeks on body mass; the levels of blood and liver malondialdehyde (MDA), creatine kinase (CK), and lactate dehydrogenase (LDH); and the serum cortisol concentrations in rats. Sprague-Dawley rats were assigned to one of three treatment groups (n=7 per group): (1) α-lipoic acid supplementation only, (2) treadmill exercise only, and (3) α-lipoic acid supplementation and exercise. Controls did not receive α-lipoic acid and did not exercise. DL-α-lipoic acid (100 mg/kg) was supplemented orally daily and rats were exercised 5 days per week for 6 weeks. The exercise regime comprised running on a treadmill at an increasing pace. After 6 weeks, body mass was significantly lower in all three treatment groups compared with controls. Liver MDA concentrations were significantly lower in the α-lipoic acid-supplemented rats, irrespective of whether the rats also exercised, than in rats that only exercised. Blood MDA and CK activities (but not LDH activity or cortisol concentrations) were significantly lower in rats that received α-lipoic acid without exercise. These results suggest that α-lipoic acid supplementation may reduce exercise-induced oxidative tissue damage via antioxidant effects.

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Key words: Malondialdehyde - DL-α-lipoic acid - Membrane damage - Oxidative stress

Introduction

Exercise and nutrition improve health by reducing the debilitating effects of various external stressors. Most physical activity increasing energy metabolism, is

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associated with an increase in oxygen consumption, and stimulates oxidation within active skeletal muscle. However, strenuous physical activity can disrupt homeostasis in unfit individuals, and exercise can thus act as a stressor that has negative effects on health. Bank *et al.* [5] reported that the negative effects of intense exercise were attributable to oxidative stress. In addition, temporary or chronic overload training or isokinetic exercise can cause muscle damage and impair metabolism [22].

Creatine kinase (CK) and lactate dehydrogenase (LDH) are, non-plasma specific enzymes that are released into blood as the result of the destruction of the cell membrane by oxidative stress or tissue damage [12,30,35], as well as the direct destruction of the cell wall and tissue necrosis [15]. Therefore, changes in the activity of CK and LDH can be used as indexes of membrane damage resulting from oxidative stress or tissue damage [6]. Recently, CK and LDH were linked to reactive oxygen species (ROS) because these enzymes are activated when cells are damaged during inflammation [7] and oxidative stress, owing to prostaglandins (PGE_2) or tumor necrosis factor-α [33]. The activity of CK and LDH increase in blood in response to exercise [37].

The reduction of the negative effects of exercise resulting from oxidative damage would enhance the benefits of exercise. Supplementing with antioxidants is a possible means of reducing exercise-induced oxidative damage. DL-α-lipoic acid is a short-chain fatty acid [38] that is analogous to vitamins that act as coenzymes for mitochondrial pyruvate or α-ketoglutarate dehydrogenase [11]. In addition, DL-α-lipoic acid acts as an antioxidant [3,4] by increasing levels of glutathione [2] and protects against DNA damage caused by stress-related proteins [19]. Few studies have addressed the effects of α-lipoic acid supplementation on oxidative tissue damage attributable to exercise. In the present study, we examined the effects of α-lipoic acid supplementation on body mass and various indexes of oxidative tissue damage in rats that underwent regular exercise for 6 weeks.

Materials and Methods

Animals and experimental protocol: Twenty-eight 8-week-old male Sprague-Dawley rats (270±10 g) were adapted for 2 weeks to the laboratory environment (22–24°C, 60% relative humidity, 12:12 light:dark photoperiod). All animals were given water and food *ad libitum*, and were treated according to the guidelines for the care and use of animals published by the National Institutes of Health. Rats were assigned randomly to one of three groups (n=7 per group): (1) α-lipoic acid supplementation only, (2) exercise only, and (3) α-lipoic acid supplementation and

exercise. Controls (n=7) did not receive α-lipoic acid and did not exercise.

The rats were exercised on rodent treadmills 5 days per week for a total of 6 weeks, under conditions of progressively increasing load. The first week was used to adapt the rats to exercise (10 min/day at 10 m/min, grade 0%). From week 2 to 4, the rats were exercised for 20 min/day at 20 m/min (grade 5%). For weeks 5 and 6, the rats were exercised for 40 min/day at 28–30 m/min (grade 5%).

DL-α-lipoic acid (Sigma, St. Louis, MO, USA) was dissolved in corn oil and was supplemented daily per on at 100 mg/kg at the same time of day for 6 weeks [1]. At the end of week 6, all rats were anesthetized with xylazine and ketamine before blood (6 ml) was withdrawn from the heart. The liver was removed and stored at –70°C.

Measurement of body mass and indexes of oxidative stress: Body mass was measured daily, at the same time each day. For the MDA analysis, protein was removed from blood serum and homogenized liver tissue by adding 15 µl (50% w/v) trichloroacetic acid to each sample, centrifuging the samples at 10.000 rpm for 10 min, and harvesting the liquid phase. Thereafter, thiobarbituric acid (TBA) (1.3% w/v) dissolved in 50 µl 50% (w/v) trichloroacetic acid, 10 µl 1 mM butylated hydroxytoluene (BHT), and 0.3% NaOH was added to the samples, which were then incubated at 60°C in a water bath for 40 min. SDS (10 µl) was then added before the optical density of each sample was measured at 530 nm using an enzyme-linked immunosorbent assay (ELISA; Biolog., USA). The standard was 1,1,3,3,-tetraethoxypropane. LDH activity was measured at 570 nm with a spectrophotometer (Amersham Pharmacia, UK), using a commercially available LDH assay kit (Boehrineger Mannheim, Germany). CK activity in serum was measured at spectrophotometrically at 340 nm, using the Helena Laboratories REP CK Isoenzyme kit. Cortisol was extracted from serum (1 ml samples) by adding dexamethasone (457 pmol) and dichloromethane to each sample tube and shaking the tubes for 20 min. The extract was washed with 2 ml NaOH (0.1 M/L) before being washed two or three times with 1 ml distilled water. The extract was then mixed with 1 g anhydrous sodium sulphate. The solvent was evaporated under a stream of nitrogen gas at 40°C, and the resulting extract was analyzed by high performance liquid chromatography (Young Lin Instrument Co., Korea).

Statistical analyses: All data were analyzed using commercially available software (SAS Institute Inc., Cary, NC, USA). We used a one-way ANOVA followed by Duncan's multiple range *post hoc* test to compare data among groups. We took $p<0.05$ to indicate statistical significance.

Table 1
The effect of exercise and α-lipoic acid supplementation on body mass during the experimental period

	Body mass (g)					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
CON	339.17±9.6[a]	363.41±10.6[a]	392.24±11.5[a]	414.23±9.7[a]	439.34±9.1[a]	470.97±9.8[a]
LAG	308.20±5.9[***b]	324.44±7.0[***b]	342.09±6.8[***b]	357.73±6.6[***b]	371.30±6.6[***b]	384.91±6.8[***b]
TRG	308.33±3.7[***b]	323.96±6.7[***b]	339.37±8.0[***b]	353.51±7.0[***b]	361.90±8.7[***c]	374.66±7.8[***c]
LTG	301.71±3.9[***b]	314.40±3.7[***c]	326.73±6.1[***c]	340.39±6.9[***c]	353.14±6.7[***d]	363.83±7.6[***d]

CON, control group; LAG, α-lipoic acid-supplemented group; TRG, treadmill-exercised group; LTG, α-lipoic acid
supplementation and treadmill exercise group

Results

The body mass in each group during the experimental period is presented in Table 1. All rats exhibited a consistent increase in body mass over the 6-week period. However, the relative increases in body mass at each week of the experiment were significantly different among the treatment groups. Specifically, the body mass was significantly lower in each of the experimental groups versus the controls. The increase in body mass during the experimental period was similar between rats treated only with α-lipoic acid and rats that were only exercised, and this increase was smaller than the body mass increase in the controls. Animals that were treated with α-lipoic acid and exercised had the smallest increase in body mass among all the groups.

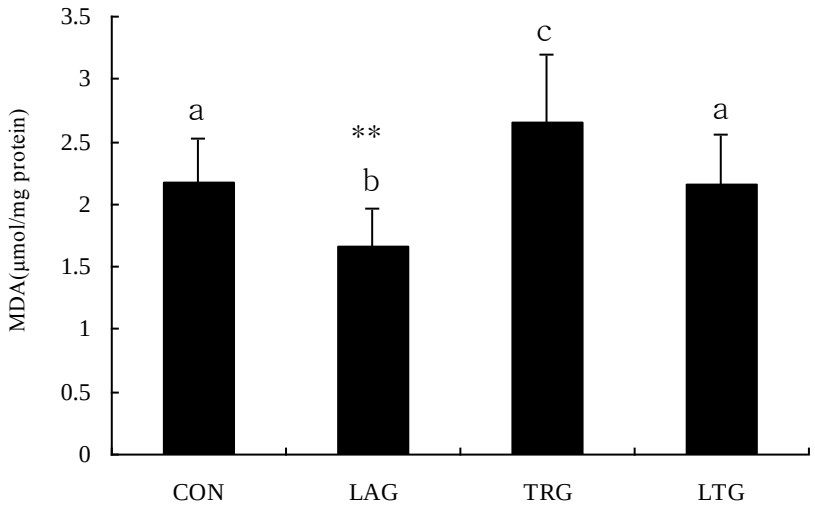

Fig. 1
The effect of exercise and α-lipoic acid supplementation on blood malondialdehyde (MDA) concentrations at the end of the experimental period.
CON, control group; LAG, α-lipoic acid-supplemented group; TRG, treadmill-exercised group; LTG, α-lipoic acid supplementation and treadmill exercise group. Lowercase letters indicate values that are not significantly different by *post hoc* analysis of ANOVA.
**Significant difference among groups ($p<0.01$)

The MDA concentrations in blood and liver tissue at the end of the experimental period, presented in Figs. 1 and 2, respectively, were significantly different among the treatment groups. Specifically, the blood MDA concentrations were significantly lower in the group treated with α-lipoic acid than in the other groups, while the liver MDA concentrations were significantly lower in the groups treated with α-lipoic acid and treated with α-lipoic acid and exercised than in the exercise-only group.

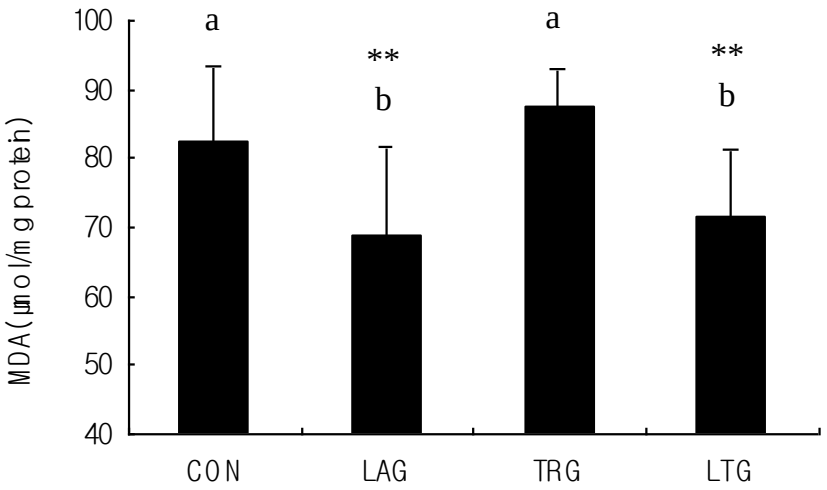

Fig. 2
The effect of exercise and α-lipoic acid supplementation on liver MDA concentrations.
CON, control group; LAG, α-lipoic acid-supplemented group; TRG, treadmill-exercised group; LTG, α-lipoic acid supplementation and treadmill exercise group. Lowercase letters indicate values that are not significantly different by *post hoc* analysis of ANOVA.
**Significant difference among groups ($p<0.01$)

The changes in CK activity are presented in Fig. 3. CK activity was significantly different among the groups. Specifically, CK activity was significantly lower in the α-lipoic acid-supplemented group compared with the other groups.

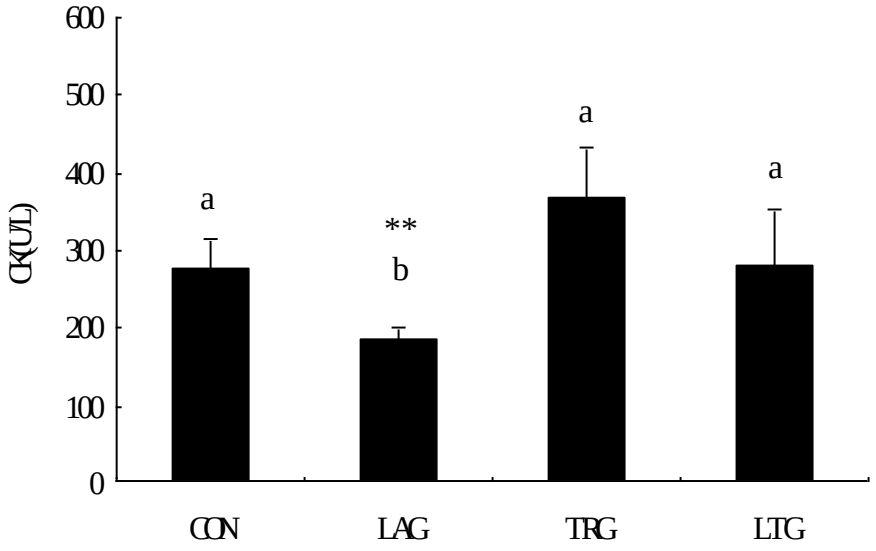

Fig. 3
The effect of exercise and α-lipoic acid supplementation on creatine kinase activity. CON, control group; LAG, α-lipoic acid-supplemented group; TRG, treadmill-exercised group; LTG, α-lipoic acid supplementation and treadmill exercise group. Lowercase letters indicate values that are not significantly different by *post hoc* analysis of ANOVA.
**Significant difference among groups ($p<0.01$)

There were no significant changes in LDH activity or cortisol concentrations in any of the treatment groups (Table 2).

Table 2
The effect of exercise and α-lipoic acid supplementation on lactate dehydrogenase (LDH) activity and serum cortisol concentrations at the end of the experimental period

	CON	LAG	TGR	LTG
LDH (U/L)	884.43±90.75	808.71±274.93	917.29±255.98	880.43±179.38
Cortisol (μg/dl)	0.37±0.09	0.34±0.14	0.42±0.06	0.41±0.09

CON, control group; LAG, α-lipoic acid-supplemented group; TRG, treadmill-exercised group; LTG, α-lipoic acid supplementation and treadmill exercise group.

Discussion

In the present study, exercise and α-lipoic acid supplementation for 6 weeks reduced the increase in body mass that occurred in control rats. Notably, the reduction in the increase in body mass of rats that received α-lipoic acid but did not exercise was similar to that of rats that exercised in the absence of α-lipoic acid supplementation. These findings suggest that α-lipoic acid can prevent increases in body mass independent of exercise. In a study of 10-week-old obese diabetic rats, Song *et al.* [32] found that α-lipoic acid suppressed food intake, promoted energy metabolism, increased the oxidation ratio of fatty acids, and promoted the expression of uncoupling protein-1 mRNA in brown adipose tissue, all of which reduce obesity. As α-lipoic acid is taken up rapidly into the CNS [28], it is likely that the α-lipoic acid-induced suppression of increases in body mass reflects a direct effect on the hypothalamus, which regulates appetite directly and also promotes a reduction in body mass by increasing the oxidation of fatty acids.

The rate of lipid peroxidation fluctuates in association with the intensity and mode of exercise. For example, a brief period of high-intensity exercise can accelerate lipid peroxidation due to oxidative stress [20,25,36], whereas long-term aerobic training can decrease lipid peroxidation and protect against tissue damage [8,13,16]. In addition, the supplementary intake of antioxidants can affect the level of lipid peroxidation. Malondialdehyde (MDA) is a byproduct of the oxidative degradation of lipids in cell membranes, and the change in MDA concentration can

be used as an index of oxidative cell damage. In the present study, the MDA concentration in the liver was lower in rats that received α-lipoic acid, irrespective of whether the rats also exercised. In contrast, the MDA concentrations in blood were lower only in α-lipoic acid-supplemented rats. Therefore, α-lipoic acid alone would appear to suppress lipid peroxidation in response to oxidative stress. This result accord with previous reports that α-lipoic acid decreased lipid peroxidation in aged mice, reduced the density of MDA and 4-hydroxyalkenal, and decreased F_2-isoprostane levels [3,26].

Several studies have demonstrated that long-term aerobic training can improve defenses against oxidative stress and can suppress lipid peroxidation. However, the facts that MDA concentrations in liver were lower in α-lipoic acid-supplemented rats irrespective of whether the rats exercised and MDA concentrations in blood were lower only in α-lipoic acid-supplemented rats suggest that the effect of exercise on peroxidation in the present study was not as great as that of α-lipoic acid. Therefore, it is possible that low- or medium-intensity exercise improves defenses against oxidative stress better than high-intensity exercise does.

CK and LDH are highly inducible enzymes that are released into the blood following oxidative damage of cell membranes or tissue injury during intense exercise, and changes in their activity in blood can be used as an indirect index of muscle damage [23,30]. For instance, the activity of CK and LDH increases in myocardium and skeletal muscle when these enzymes are activated as a result of damage to the sarcolemma that follows the exhaustion of glycogen. The release of CK and LDH into the blood and tissue is the result of increased permeability of the plasma membrane due to lipid peroxidation [30]. CK and LDH have also been linked to ROS, because the activity of these enzymes is related to cell damage during inflammation [7] cell proliferation, and oxidative stress [33]. The activities of CK and LDH is closely related to that of MDA. This was indicated by the findings of Kim *et al.* [17], who reported that LDH and MDA activity in rats that exercised until exhaustion were highest after the exercise. Kanter *et al.* [14] and Krotkiewski *et al.* [21] also reported that exercise-induced increases in MDA concentrations in the blood were correlated with an increase in activity. In the present study, CK activity were significantly higher in rats that exercised, but were significantly lower in rats that received α-lipoic acid. Therefore, α-lipoic acid may protect the cell membrane against exercise-induced oxidative damage by suppressing the release of CK into the blood. This is analogous to the findings of several earlier studies. Rokitzki *et al.* [29] reported that the administration of vitamins E and C (both of which are antioxidants) caused a decrease in CK activity in the blood. Similarly, Sumida *et al.* [34] reported that administering vitamin E to

healthy young men prior to intensive exercise decreased both CK activity and the concentration of MDA. Finally, McBride *et al.* [24] found that CK activity in the blood were lower after 24 h of exercise following the intake of vitamin E.

The suppression of lipid peroxidation by α-lipoic acid may be attributed to a blockade of the radical-initiated oxidative chain reaction and/or the resynthesis of antioxidants such as glutathione and vitamin C [27,31]. DL-α-lipoic acid is found in human and animal tissue, where it is a latent antioxidant that mainly suppresses lipid peroxidation following oxidative stress and improves the levels of other antioxidants [3,4]. DL-α-lipoic acid not only increases the production of glutathione [10,31] but also enhances the resynthesis of vitamins C and E, thereby effectively eliminating potentially damaging ROS such as hydroxyl, peroxyl, and superoxide radicals [27]. Dihydrolipoic acid, which is the reduced form of α-lipoic acid, reduces the levels of hydroxyl radicals generated by iron [9]. In addition, dihydrolipoic acid can cross the blood-brain barrier to eliminate radicals generated by the oxidation of dopamine, serotonin, and norepinephrine [4], and can also prevent the oxidative damage at kidney and endothelial cells that occurs during diabetes [26]. Recently, it was demonstrated that α-lipoic acid can eliminate superoxide anions and that this process depends on both the concentration of α-lipoic acid and pH [38].

In the present study, LDH activity was not significantly affected by α-lipoic acid supplementation or exercise. This result is contrary to the findings of Kim et al. [18], who reported that intense exercise (35 m/min, 5% grade, 30 min) following α-tocopherol acetate supplementation did not alter total LDH activity but did change the activity of LDH isozymes. This discrepancy might result from differences in the experimental methods and/or differences in the effects of α-lipoic acid and α-tocopherol acetate on the activities of different LDH isozymes.

In conclusion, we found that α-lipoic acid supplementation reduced MDA and CK activities. Therefore, α-lipoic acid supplementation may have beneficial antioxidant effects that reduce tissue damage during exercise-induced oxidative stress.

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