EFFECTS OF $\alpha$-LIPOIC ACID SUPPLEMENTATION ON ENDURANCE CAPACITY AND ENERGY METABOLISM IN RATS

Tatsuhiro MATSUO and Kanako YAGI

Abstract

$\alpha$-Lipoic acid (ALA) functions as a cofactor of mitochondrial enzymes, is closely related with mechanism of aerobic energy production. Therefore, ALA is expected to provide improvement of endurance capacity and recovery of fatigue, but it is not well known that ALA has effect of energy metabolism. In this study, we investigated the promoting effects of ALA on energy metabolism in rats, used exercise time to exhaustion in loading swimming test and energy expenditure before and after exercise as indicators. Male Wistar rats were orally administrated $100\text{mg/kg}$ body weight of ALA for 4 weeks. The effects of ALA on exhaustion time were not observed, while resting metabolic rate was significantly increased by daily administration of ALA. Moreover, ALA increased total energy expenditure of the post-exercise, but the difference was not significant. These results suggest that ALA does not enhance endurance, but promotes activation of energy metabolism.

Key words: $\alpha$-Lipoic acid; endurance capacity; energy metabolism; rat; swimming

Introduction

$\alpha$-Lipoic acid (ALA) occurs endogenously in tissues and functions as a mitochondrial coenzyme for enzymatic reactions involved in the oxidative decarboxylation of $\alpha$-keto acids (pyruvate dehydrogenase, $\alpha$-ketoglutarate dehydrogenase), is closely related with mechanism of aerobic energy production.\(^1\) It has been used as therapy for many diseases associated with impaired energy utilization. Dietary supplementation of ALA can act as a potent antioxidant and ameliorate oxidative stress both in vitro and in vivo.\(^2\) Therefore, ALA is expected to promote energy production, provide to improvement of endurance capacity and recovery of fatigue.

Hagen et al.\(^3\) reported that ALA supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. These results suggested that ALA supplementation elevated energy production. However, research reports about the promoting effect of ALA on energy metabolism in vivo are limited, and it has not been known whether ALA supplementation influence energy metabolism during exercise. In this study examined the effects of ALA on endurance capacity and energy metabolism in rats. We used exercise time to exhaustion in loading swimming test and energy expenditure before and after exercise as indicators of endurance capacity and energy metabolism.

Materials and Methods

All procedures involving animals were approved by the Experimental Animal Care Committee of the Kagawa University.

Animals and experimental design

Thirty-nine male Wistar rats (3 weeks old) were purchased from Japan SLC, Inc. (Shizuoka) and were acclimatized for a week under standard laboratory conditions (22 ± 1°C, 50-60% humidity). The light/dark cycle was 12h with lights on from 8:00h to 20:00h. After the adaptation of a week, all rats were randomized by body weight to two groups. One group was a control (C, n = 20) and the other was $\alpha$-lipoic acid group (LA, n = 19). Both groups were fed 5g of commercial rat chow (CE-2, Japan CLEA, Inc., Tokyo) twice a day (8:30-9:30h, 20:30-21:30h). All rats fed the diets isoenergetically during 4 weeks (from 4 to 8 weeks old) of experimental period. The LA group was administered orally $\alpha$-lipoic acid (100mg/kg/day) dissolved in 0.2% of NaOH in physiological saline and the C group was administered orally 0.9% physiological saline at 10:30h.

Exercise time to exhaustion in loading swimming test

At the age of 6 weeks, all rats were forced to swim with loads equal to 6% of body weight attached to chest, and the
swimming time until fatigue was measured in all rats. A time point until fatigue was defined as the failure to rise to the surface of the water to breathe or not to move limbs below water by a modification described by Matsumoto et al. \(^{9}\). At 30, 60, 90, and 120 min after loading test, blood was collected from the tail vein to obtain plasma for analysis of plasma lactic acid concentrations. Plasma lactic acid concentration was determined by enzyme method used lactate dehydrogenase by a modification described by Gutmann et al. \(^{10}\) and Wieland et al. \(^{11}\).

**Energy metabolism measurement**

Energy expenditure before and after exercise was measured by the breath-by-breath method, using a respiratory gas analyzer (LX-710 and G-102, Iizima Electronics Industry Corporation, Aichi, Japan) at the age of 7 weeks in all rats. The measurements of resting energy metabolism were carried out for 10 min. After all rats were forced to swim for 1h with loads equal to 4% of body weight attached to chest. Before measurements, all rats were housed quietly for 30 min in metabolimeter to adjust to measurement environment. After swimming, the measurements were carried out for 3.5h during the resting state. Respiratory quotient (RQ) and energy expenditure (EE) were calculated by using equations described by Weststrate. \(^{12}\)

\[
RQ = \frac{\dot{V}CO_2}{\dot{V}O_2}
\]

\[
EE (\text{kJ/min}) = 4.184 \left(\frac{4.686 + 1.096 (RQ - 0.707)}{\dot{V}O_2}\right)
\]

**Dissection of animals**

At the end of experiment (8 weeks old), each group of rats was randomly assigned by body weight to two sub groups, the groups were killed before and after exercise. All rats swam for 30 min at 14:00 to adjust swimming exercise during experimental period. We used an plastic pool (80 x 56 x 48 cm) filled to a depth of 40 cm with 30°C water. The pre-exercise group was killed by decapitation 3.5h after administration (at 14:00h). The post-exercise group was forced to swim for 1h (from 14:00h to 15:00h) with loads equal to 4% of body weight attached to chest, and after the swimming load, rats were killed by decapitation immediately. Blood was collected to obtain serum, and liver, gastrocnemius and soleus muscles were removed immediately, frozen in liquid nitrogen, and kept at -80°C until analysis.

**Biochemical examination**

Gastrocnemius muscle lactic acid content was determined by enzyme method used lactate dehydrogenase by a modification described by Gutmann et al. \(^{10}\) and Wieland et al. \(^{11}\). Briefly, the muscle sample was added to 0.8N perchloric acid solution, then homogenized and centrifuged (14,000rpm, 1 min). The supernatant obtained was determined. Gastrocnemius muscle lactic acid dehydrogenase activity was determined using the supernatant obtained via homogenized together with 0.1mol/L Tris-HCl buffer and centrifuged (14,000rpm, 1 min) by a commercial kit (lactate dehydrogenase C II -test Wako, Wako pure chemical Industries, Ltd., Osaka). Gastrocnemius and Soleus muscles glycogen content were determined by a modification described by Lo et al. \(^{13}\). Serum and liver lipid peroxidation were determined by a modification described by Ohkawa et al. \(^{14}\).

**Statistical analysis**

All values were expressed as mean ± SD. Statistical analysis of differences between C and LA groups were performed with Student’s t-test. Comparisons of the biochemical analysis in each group were performed by two-way ANOVA and Fisher’s PLSD tests. Statistical significance was set at p value of <0.05. All analyses were performed with a commercially available statistical package (StatView J-5.0, SAS Institute Inc., Cary, NC).

**Results**

**Exercise time to exhaustion in loading swimming test**

Exhaustion time in loading swimming test did not differ between the C and LA groups, as shown in Fig. 1. After 90 min swimming, plasma lactic acid concentration was significantly lower in the LA group than in the C group, however at 30, 90 and 120 min after swimming, plasma lactic acid concentrations did not differ between two groups, as shown in Fig. 2.

**Energy metabolism measurement**

Resting metabolic rate (RMR) was significantly increased by ALA administration, and post-exercise energy expenditure during 3.5h was greater in the LA group than in the C group, however the difference was not significant (Table 1, Fig. 3.). Pre- and post-exercise RQ did not differ between the C and LA groups, as shown in Fig.4.

**Biochemical examination** (Table 2)

Lactic acid content of gastrocnemius muscle in the pre-exercise did not differ between the C and LA groups, but in the
post-exercise, it was significantly lower in the LA group than in the C group. Lactate dehydrogenase activity of gastrocnemius muscle in the pre-exercise did not differ between the C and LA groups, but in the post-exercise, it was significantly higher in the LA group than in the C group. Glycogen contents of gastrocnemius and soleus muscles in the pre-exercise were significantly increased by ALA administration, but in the post-exercise, they did not differ between the C and LA groups. Serum and liver lipid peroxidation concentrations in both the pre- and post-exercise were significantly lower in the LA group than in the C group.

Discussion

In this study, we demonstrated that ALA continuously-administered had no effect on improvement of endurance capacity of rats, but had effect on a significantly increasing resting metabolic rate. We also showed that energy expenditures after exercise increased but not significant by ALA continuously-administered. These results suggested ALA administration...
increased energy metabolism in rats.

ALA functions as a cofactor of mitochondrial enzymes, is closely related with mechanism of aerobic energy production. Because of essential biogenic factor for promoting metabolism, it is not difficult to understand that ALA continuously-administered markedly enhanced energy expenditure in rats. Recent study reported that ALA suppresses AMP-activated protein kinase (AMPK) in the hypothalamus but activates it in skeletal muscle. AMPK functions as a fuel sensor in the cell and is activated when cellular energy is depleted. Lee et al. recently reported that ALA increased fatty acid oxidation in skeletal muscle by activating AMPK. AMPK activation increases fatty acid oxidation by inhibiting acetyl-coenzyme A (acytly-CoA) carboxylase (ACC) activity and by decreasing malonyl-CoA concentrations. In this study, we were not observed that respiratory quotient increased by ALA-administrated. Therefore, it might be possible that ALA increases energy expenditure by the other mechanisms. In rodents, uncoupling protein (UCP)-1 in brown adipose tissue is the main regulator of basal energy expenditure, and the expression of this protein is increased by adrenergic stimulation. Lee et al. had shown that central administration of very small amounts of ALA increased expression of UCP-1 mRNA in brown adipose tissue and energy expenditure in whole body.

We demonstrated that ALA induced significant increase of muscle glycogen accumulation after exercise and decrease of muscle lactic acid accumulation after exercise. ALA has been used as therapy for many diseases associated with impaired energy utilization, such as type II diabetes and diabetic polyneuropathies. This has been shown to protect against oxidative stress-induced insulin resistance in vitro and to increase insulin-stimulated glucose uptake in skeletal muscle by improving insulin sensitivity. In skeletal muscle, glucose transport can be activated by two independent mechanisms, one is insulin-dependent and the other is insulin-independent. Recently, several studies have demonstrated that ALA can directly activate glucose transport by activation of AMPK. In this study, ALA enhanced skeletal muscle glycogen synthesis, expecting that glucose uptake in skeletal

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<th>Pre-exercise</th>
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<td></td>
<td>C</td>
<td>LA</td>
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<tr>
<td>Gastrocnemius lactate acid (mg/g tissue weight)</td>
<td>62.0 ± 15.0</td>
<td>60.8 ± 10.6</td>
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<td>Gastrocnemius lactate dehydrogenase (IU/g tissue weight)</td>
<td>756 ± 22</td>
<td>757 ± 33</td>
<td>688 ± 46</td>
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<tr>
<td>Gastrocnemius glycogen (mg/g tissue weight)</td>
<td>3.986 ± 0.96</td>
<td>4.771 ± 0.87</td>
<td>2.011 ± 0.50</td>
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<td>Soleus glycogen (mg/g tissue weight)</td>
<td>0.781 ± 0.24</td>
<td>1.384 ± 0.33</td>
<td>0.055 ± 0.02</td>
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<td>Serum lipid peroxidation (nmol MDA equivalent/ml)</td>
<td>6.13 ± 0.29</td>
<td>5.00 ± 0.61</td>
<td>11.93 ± 1.40</td>
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<tr>
<td>Liver lipid peroxidation (mol MDA equivalent/g tissue weight)</td>
<td>249 ± 33</td>
<td>211 ± 24</td>
<td>363 ± 45</td>
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Values are expressed as the mean ± SD for 9-10 rats. Means with different superscripts within a row are significantly different (p<0.05, two-way ANOVA and Fisher’s PLSD tests).

Fig. 4 Change in RQ before and after exercise. Values are expressed as the means and SD for 19-20 rats. C, control group ; LA, α-Lipoic acid group.
muscle was promoted. In addition, this study implied that glycolytic system was inhibited because ALA was reported to enhance fatty acids oxidation. This could lead to accumulation of glycogen and reduction of lactic acid contents in skeletal muscle. The importance of muscle glycogen on endurance performance has been demonstrated by the study reported that endurance capacity depended on accumulation of muscle glycogen. In contrast, it has been shown that lactic acid, metabolite formed by glycolytic system, decreased by endurance training, resulting that lactic acid utilization increased as the energy source. In this study, ALA reduced lactic acid contents in skeletal muscle after swimming. This finding suggests that ALA produces the same effects as endurance training, or ALA enhances the efficiency of training.

When much oxygen is taken into the body during exercise, level of oxygen in the body's tissues increase, and it followed that lipid peroxidative reactions are induced in blood, skeletal muscle, myocardium and liver. There is strong evidence that exercise-induced oxidative stress decreases exercise performance. Some researchers indicated that ALA, a potent antioxidant, markedly reduced oxidative stress. In this study, serum and liver lipid peroxidation concentrations in both before and after exercise were significantly lower by ALA administration. Our results at least in part support previous finding and ALA reduces oxidative damage, expecting to enhance exercise performance.

In conclusion, we demonstrated in this study that ALA did not contribute directly to enhance endurance capacity, but promoted accumulation of muscle glycogen and decreased accumulations of lactic acid and lipid peroxidation after exercise. These results suggest that ALA supplementation leads to enhance the efficiency of training and reduce fatigue and biological oxidation damage by exercise. Thus, ALA may be useful as a supplement for athletes.

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T. MATSUO, et al. : Effects of \( \alpha \)-lipoic acid on energy metabolism


(Received October 31, 2007)

\( \alpha \)-リポ酸投与がラットの持久性運動能力とエネルギー代謝に及ぼす影響

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\( \alpha \)-リポ酸はミトコンドリアに存在する酸化系酵素の補酵素であり、有酸素系エネルギー代謝に深く関わっている。それゆえ、\( \alpha \)-リポ酸摂取が持久性運動能力向上と疲労回復をもたらすと期待されている。しかしながら、\( \alpha \)-リポ酸のエネルギー代謝系に及ぼす影響については、あまり検討されていない。そこで本研究では、ウイスター系雄ラットに100mg/kg体重の\( \alpha \)-リポ酸を4週間経口投与し、\( \alpha \)-リポ酸がエネルギー代謝に及ぼす影響をラットのスイミング運動持続時間、運動前後のエネルギー消費量を指標として検討した。\( \alpha \)-リポ酸投与により、安静時のエネルギー消費量は有意に増加し、運動後のエネルギー消費量も増加する傾向が見られたが、スイミング運動持続時間の延長は確認できなかった。これらの結果から、\( \alpha \)-リポ酸は、運動能力の向上には寄与しないが、エネルギー代謝を増加させることが示唆された。