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## ASSESSMENT OF THE ANTIOXIDANT EFFECTIVENESS OF $\alpha$ -LIPOIC ACID IN HEALTHY MEN EXPOSED TO MUSCLE-DAMAGING EXERCISE

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The aim of this study was to compare the indices of glutathione antioxidant system and oxidative damage level in resistance trained and untrained subjects and to assess the antioxidant action of  $\alpha$ -lipoic acid in trained men exposed to muscle-damaging exercise. Thirteen trained and twenty untrained men (NT) participated in the comparative study. Then trained men were randomly assigned to T<sub>CON</sub> group (control) or T<sub>ALA</sub> group ( $\alpha$ -lipoic acid, 600 mg · day<sup>-1</sup>, for 8 days) and performed isometric/isokinetic effort of quadriceps muscles. The study has shown the significantly higher erythrocyte levels of glutathione (GSH), glutathione reductase (GR) and glutathione peroxidase (GPx) in T<sub>CON</sub> than NT but no differences in plasma lipid peroxidation (TBARS) and protein carbonylation (PC). However, total thiol (TT) concentration was two-fold lower in T<sub>CON</sub> than NT group.  $\alpha$ -Lipoic acid variously influenced the post-exercise levels of GSH (+40%), GR (-24%) and GPx (+29%), but markedly reduced by over 30% the resting and post-exercise TBARS and PC in T<sub>ALA</sub> compared with T<sub>CON</sub>. TT concentration significantly increased in T<sub>ALA</sub> but it did not reach the high level which was found in untrained group. It is concluded that  $\alpha$ -lipoic acid supplementation diminishes oxidative damage. It does not abolish differences in glutathione antioxidant system between untrained and trained subjects but modulates a pro-antioxidant response to the muscle-damaging exercise.

**Key words:** *thiols, antioxidant enzymes, oxidative damage, isokinetic dynamometer*

### INTRODUCTION

It has been generally accepted that regular and intense exercises lead to reactive oxygen and nitrogen species (RONS) generation and changes in pro-antioxidant parameters such as glutathione, glutathione enzymes, lipid peroxidation products *etc.* Therefore the measurement of various pro-antioxidant parameters can be used in determining risk of oxidative stress or effectiveness of antioxidant supplementation.

$\alpha$ -Lipoic acid (ALA) as a pro-glutathione dietary supplement has been the focus of intensive research in nutrition in the last few years. ALA and its reduced form dihydrolipoate (DHLA) have been suggested to function as powerful antioxidant. ALA and DHLA couple has shown the ability to react with reactive and oxygen species (RONS) such as hydroxyl radical, hypochlorous acid and singlet oxygen and reduce glutathione disulfide, tocopherol radicals and ascorbate. Moreover, DHLA and ALA can work as a redox regulator of myoglobin, prolactin, thioredoxin, glucose transporter protein (GLUT4) and NF- $\kappa$ B transcription factor. ALA, as lipoaamid, has functioned as a cofactor in the multienzyme complexes that catalyse the oxidative decarboxylation of  $\alpha$ -keto acids such as pyruvate,  $\alpha$ -ketoglutarate, and branched chain  $\alpha$ -keto acids (1-3).

Even though, ALA has been applied in sport as a dietary supplement, its use by the athletes gives rise to controversy. It has been reported that ALA supplementation prevented the decline of

others antioxidants, improved glucose metabolism and attenuated exercise-induced oxidative damage in various tissues (1). On the other hand, it has been shown that long-term ALA administration led to enhancement of lipid peroxidation, mitochondrial damage and inhibition of glycogen synthesis (1, 2). The majority results concerning the effects of ALA on cell metabolism have been obtained from *in vitro* studies or animal models (4-9). There has been lack of studies in physically active men.

Thus, the aim of the present study was 1) to compare the parameters of pro-antioxidant status in untrained and trained healthy men, and 2) to assess the antioxidant effectiveness of oral  $\alpha$ -lipoic acid administration in trained men performed muscle-damaging exercise.

### MATERIALS AND METHODS

Thirteen healthy and weight trained males with resistance training experience of at least 3 years (T group: age 25.5  $\pm$  6.0 yr, height 180.5  $\pm$  5.9 cm, body mass 86.4  $\pm$  8.1 kg, body fat 14.1  $\pm$  3.9%) participated in this randomised, double-blind, placebo-controlled and cross-over study. Twenty healthy, non-smoking and untrained males (NT group: age 21.8  $\pm$  1.1 yr, height 180.3  $\pm$  7.8 cm, body mass 82.2  $\pm$  8.7 kg, body fat 12.5  $\pm$  4.4%) made a reference group. Subjects had not taken any supplements, particularly antioxidant vitamins or minerals, for 4 weeks prior

to the study. All the subjects were informed of the aim of the study and were given their written consent for participation in the project. The protocol of the study was approved by the local ethics committee in accordance with the Helsinki Declaration.

Trained (T) subjects were administered with 600 mg of  $\alpha$ -lipoic acid ( $T_{ALA}$  group) or 700 mg lactose (placebo,  $T_{CON}$  group) for eight days before the muscle-damaging effort (isometric/isokinetic exercise). The participants took the  $\alpha$ -lipoic acid or placebo in the morning in a fasted state. The last dose of ALA was taken 24 h before exercise. The wash-out period between the trials with  $\alpha$ -lipoic acid and placebo was three weeks.

#### Blood collection

Blood samples were obtained from a cubital vein with an anticoagulant (EDTAK<sub>2</sub>) in the morning in NT group. In trained men ( $T_{CON}$  and  $T_{ALA}$ ), the blood samples were collected before exercise, immediately after completing exercise and after 24 h of the recovery period. The samples were immediately placed in 4°C temperature after collection. Within 10 min, the blood samples were centrifuged (2500 g, 10 min, 4°C). Aliquots of plasma were stored at -20°C. Erythrocyte fraction was resuspended three times in cold isotonic saline solution and centrifuged (2500 g, 10 min, 4°C). Washed erythrocytes were stored at -20°C until analysis. All the samples were analysed within 7 days.

#### Isometric/isokinetic exercise

The exercise protocol consisted of three 10-second maximal voluntary (the highest efficiency) isometric contractions of the quadriceps muscles in 30° and 75° of knee flexion on Biodex System 3 dynamometer (Biodex Medical Systems, Shirley, NY). Subjects finished the isometric sequence with peak torque for right limb on the level of 195.1 ± 28.6 Nm (extensor m.) and 154.9 ± 26.3 Nm (flexor m.) at 30° knee flexion, 357.4 ± 46.2 Nm (extensor m.) and 127.6 ± 28.4 Nm (flexor m.) at 75° knee flexion, and for left limb on the level of 198.7 ± 33.8 Nm (extensor m.) and 138.9 ± 27.1 Nm (flexor m.) at 30° knee flexion, 335.1 ± 55.0 Nm (extensor m.) and 123.7 ± 24.0 Nm (flexor m.) at 75° knee flexion. Then the isokinetic sequence was carried out at angular velocities of 60, 120, 180, 210 and 450°·s<sup>-1</sup>. Isokinetic sequence was performed in seated position with the knee in 90° of flexion and repeated until exhaustion. Peak torque, time to reach peak torque, total work, average power and max average peak torque were collected from the Biodex during muscle performance measures. Subjects were familiarized with the both exercise protocols before the start of data collection.

#### Biochemical analysis

The plasma total thiol (TT) concentration was estimated by the method of Hebeeb (10) using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). The samples were added to a denaturing solution containing sodium dodecyl sulfate in order to ionise the sulphhydryl groups and make them more reactive to DTNB. The samples were measured at 410 nm against control samples (minus DTNB). The intra-assay coefficient of variation (CV) for thiol procedure was <7%.

Blood reduced glutathione (GSH) concentration was estimated by the method of Beutler *et al.* (11) using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). GSH detection limit for the procedure was 2.5 µg · ml<sup>-1</sup> and the intra-assay coefficient of variation (CV) was <10%.

Erythrocyte glutathione reductase (GR) and glutathione peroxidase (GPx) activities were evaluated using Randox assay (UK). GR and GPx detection limits for Randox kits were 11 U · l<sup>-1</sup>

and 8.86 U · l<sup>-1</sup>, respectively. The intra-assay coefficient of variation (CV) for the GR kit was 4.63% and for the GPx kit it was 4.20%.

The enzymatic activities and glutathione concentration were expressed relatively to Hb concentration measured by the Drabkin method (12) using Drabkin's reagent (POCH Poland).

Plasma lipid peroxidation products were estimated using the measurement of thiobarbituric acid-reactive substance (TBARS) level according to the method of Buege and Aust (13). To avoid further peroxidation, plasma samples were treated with 15% TCA containing 0.25M HCl immediately after separation of plasma. The TBARS level was expressed as nmol of malondialdehyde using 1,1,3,3-tetraethoxypropane as a standard. TBARS detection limit was 0.13 nmol · ml<sup>-1</sup>.

Plasma protein carbonyls (PC) were measured by the method of Levine *et al.* (14) using 2,4-dinitrophenyl hydrazine. The carbonyl content was calculated using an extinction coefficient of 22000 M<sup>-1</sup> · l<sup>-1</sup> · cm<sup>-1</sup> and expressed as nmol PC per mg of plasma protein. Protein concentration was determined by the method of Bradford (15). The intra-assay coefficient of variation (CV) for TBARS and PC procedures were <10%.

Plasma creatine kinase (CK) and lactate dehydrogenase (LDH) activities were evaluated using the diagnostic assays for the kinetic enzyme analyser Konelap 60 BioMerieux (France). CK and LDH detection limits for the applied kits were 6 U · l<sup>-1</sup> and 18 U · l<sup>-1</sup> respectively. The intra-assay coefficient of variation (CV) for the CK kit was 1.85% and the LDH kit was 2.61%.

#### Statistics

Statistical analysis was carried out using Statistica 6.0. All the data were tested for their normal distribution. For the comparison of untrained (NT) and trained subjects ( $T_{CON}$ ,  $T_{ALA}$ ) one-way ANOVA was chosen. To determine the effects of exercise and  $\alpha$ -lipoic acid as well as the interaction between exercise and supplementation, statistical analysis was performed using two-way ANOVA and post-hoc Tukey's test. Correlations were calculated by the Pearson correlation coefficients. The accepted level of significance was defined as  $P < 0.05$ . Data are presented as mean ± SD.

## RESULTS

The comparison of pro-antioxidant parameters has indicated significant differences between untrained and trained subjects concerning blood GSH, erythrocyte GR and GPx, plasma TT but no differences in plasma TBARS and PC levels. The resting plasma TT concentration was almost two-fold higher in NT (167.10 ± 14.58 mg · l<sup>-1</sup>) than in  $T_{CON}$  (83.26 ± 10.35 mg · l<sup>-1</sup>). Eight-day ALA administration significantly elevated the resting TT level in  $T_{ALA}$  to 112.60 ± 10.82 mg · l<sup>-1</sup> which remained on high level at 48 h after the last dose of ALA (*Fig. 1*). In  $T_{ALA}$ , TT did not reach the level found in NT group. This could indicate the high thiol consumption during resistance training. The resting GSH concentration was significantly lower in NT (1.35 ± 0.13 mg · gHb<sup>-1</sup>) than in trained group ( $T_{CON}$ : 1.94 ± 0.18 mg · gHb<sup>-1</sup>). ALA administration did not affect the resting GSH concentration (*Fig. 2*). The resting activities of glutathione-related enzymes i.e. GR (NT: 14.27 ± 2.75 U · gHb<sup>-1</sup>) and GPx (NT: 39.51 ± 12.97 U · gHb<sup>-1</sup>) were considerably lower in NT than in trained subjects (*Fig. 3, 4*). ALA intake caused increase in resting GR thus elevating the difference in enzyme activities by 30% between NT and trained subjects. The resting GPx activity decreased following  $\alpha$ -lipoic acid but did not reach the level observed in NT group. TBARS and PC concentrations in NT were similar those in  $T_{CON}$  group, however, ALA significantly reduced the resting plasma lipid peroxidation and protein carbonylation (*Fig. 5, 6*).

The applied isometric/isokinetic effort induced muscle damage in trained group. This was demonstrated by statistically significant changes in plasma activities of muscle damage markers. Plasma CK activity increased from resting values of  $149.40 \pm 35.57 \text{ U} \cdot \text{l}^{-1}$  ( $T_{\text{CON}}$ ) and  $150.00 \pm 42.26 \text{ U} \cdot \text{l}^{-1}$  ( $T_{\text{ALA}}$ ) to  $418.50 \pm 161.04 \text{ U} \cdot \text{l}^{-1}$  ( $T_{\text{CON}}$ ) and  $534.25 \pm 160.06 \text{ U} \cdot \text{l}^{-1}$  ( $T_{\text{ALA}}$ ) at 24 h of the post-exercise recovery period. LDH activity increased from  $303.91 \pm 56.73 \text{ U} \cdot \text{l}^{-1}$  ( $T_{\text{CON}}$ ) and  $297.90 \pm 68.20 \text{ U} \cdot \text{l}^{-1}$  ( $T_{\text{ALA}}$ ) to  $418.45 \pm 65.81 \text{ U} \cdot \text{l}^{-1}$  ( $T_{\text{CON}}$ ) and  $381.00 \pm 77.81 \text{ U} \cdot \text{l}^{-1}$  ( $T_{\text{ALA}}$ ) at 24 h of the post-exercise period. The changes in CK and LDH activities were independent of  $\alpha$ -lipoic acid supplementation and did not correlate with oxidative damage markers.

As it was showed in Fig. 1-6, even though the isometric/isokinetic exercise did not trigger off visible changes in pro-antioxidant parameters, its application has enhanced  $\alpha$ -lipoic

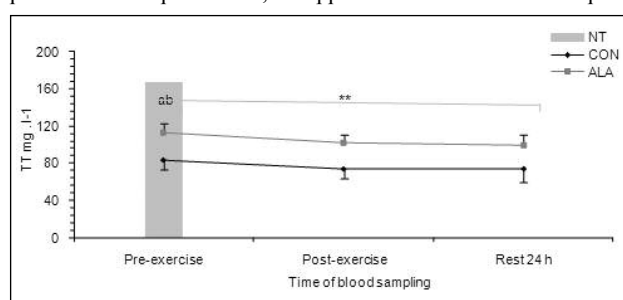


Fig. 1. Changes in plasma total thiols (TT) concentration; \*\*  $P < 0.01$  indicates  $T_{\text{CON}}$  vs.  $T_{\text{ALA}}$ ; ab means significant ( $P < 0.05$ ) difference between NT and trained subjects ( $T_{\text{CON}}$  and  $T_{\text{ALA}}$ ).

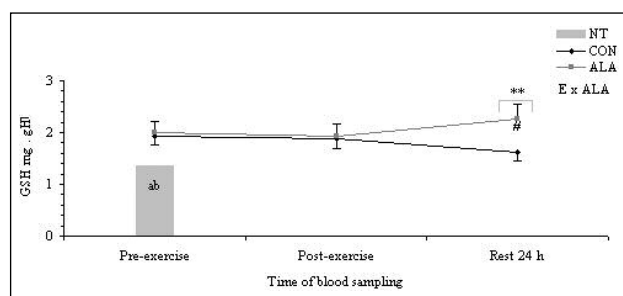


Fig. 2. Changes in blood reduced glutathione (GSH) concentration; \*\*  $P < 0.01$  indicates  $T_{\text{CON}}$  vs.  $T_{\text{ALA}}$ ; #  $P < 0.05$  indicates post-exercise vs. pre-exercise values; E x ALA indicates a significant interaction between exercise and supplementation (ANOVA,  $P < 0.05$ ); ab means significant ( $P < 0.05$ ) difference between NT and trained subjects ( $T_{\text{CON}}$  and  $T_{\text{ALA}}$ ).

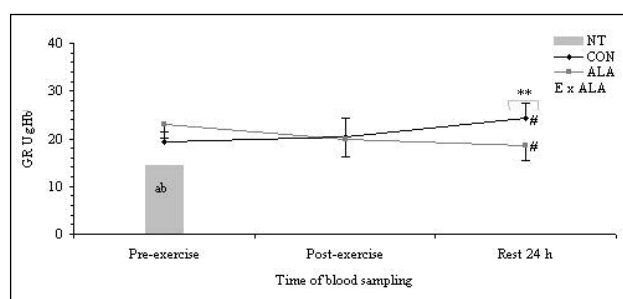


Fig. 3. Changes in erythrocyte glutathione reductase (GR) activity; \*\*  $P < 0.01$  indicates  $T_{\text{CON}}$  vs.  $T_{\text{ALA}}$ ; #  $P < 0.05$  and ##  $P < 0.01$  indicate post-exercise vs. pre-exercise values; E x ALA indicates a significant interaction between exercise and supplementation (ANOVA,  $P < 0.05$ ); ab means significant ( $P < 0.05$ ) difference between NT and trained subjects ( $T_{\text{CON}}$  and  $T_{\text{ALA}}$ ).

acid action particularly in relation to glutathione and glutathione-related enzymes. The changes in GSH, GR and GPx were demonstrated 24 h after exercise. Blood GSH concentration significantly increased from  $1.62 \pm 0.17 \text{ mg} \cdot \text{gHb}^{-1}$  in  $T_{\text{CON}}$  to  $2.26 \pm 0.28 \text{ mg} \cdot \text{gHb}^{-1}$  in  $T_{\text{ALA}}$  (Fig. 2). GR activity decreased from  $24.27 \pm 3.13 \text{ U} \cdot \text{gHb}^{-1}$  ( $T_{\text{CON}}$ ) to  $18.54 \pm 2.15 \text{ U} \cdot \text{gHb}^{-1}$  in  $T_{\text{ALA}}$  whereas GPx activity increased from  $43.03 \pm 7.65 \text{ U} \cdot \text{gHb}^{-1}$  ( $T_{\text{CON}}$ ) to  $55.30 \pm 9.99 \text{ U} \cdot \text{gHb}^{-1}$  in  $T_{\text{ALA}}$  group (Fig. 3, 4). ANOVA showed a significant interaction between ALA and exercise (Fig. 2, 4).

The levels of TT, TBARS and PC changed in  $T_{\text{ALA}}$  group independently of applied isometric/isokinetic exercise i.e. ANOVA did not show any interactions between ALA and exercise. In  $T_{\text{ALA}}$ , TT significantly increased whereas peroxidation and carbonylation products decreased before and after exercise compared with  $T_{\text{CON}}$ . The high concentration of TT and low of TBARS and PC remained to 48 h after the last dose of ALA (Fig. 1, 5, 6).

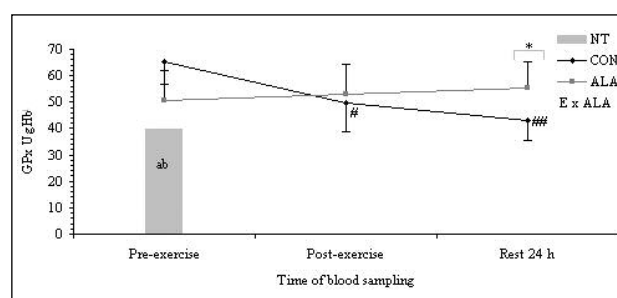


Fig. 4. Changes in erythrocyte glutathione peroxidase (GPx) activity; \*  $P < 0.05$  indicates  $T_{\text{CON}}$  vs.  $T_{\text{ALA}}$ ; #  $P < 0.05$  and ##  $P < 0.01$  indicate post-exercise vs. pre-exercise values; E x ALA indicates a significant interaction between exercise and supplementation (ANOVA,  $P < 0.05$ ); ab means significant ( $P < 0.05$ ) difference between NT and trained subjects ( $T_{\text{CON}}$  and  $T_{\text{ALA}}$ ).

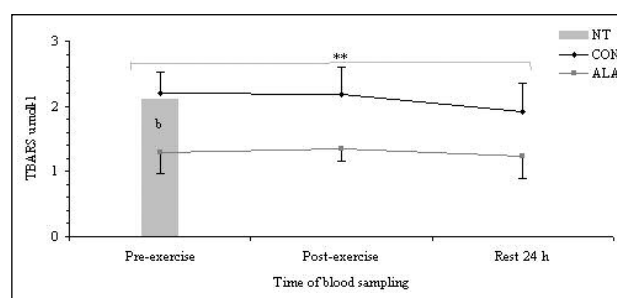


Fig. 5. Changes in plasma lipid peroxidation (TBARS) concentration; \*\*  $P < 0.01$  indicates  $T_{\text{CON}}$  vs.  $T_{\text{ALA}}$ ; b means significant ( $P < 0.05$ ) difference between NT and  $T_{\text{ALA}}$  groups.

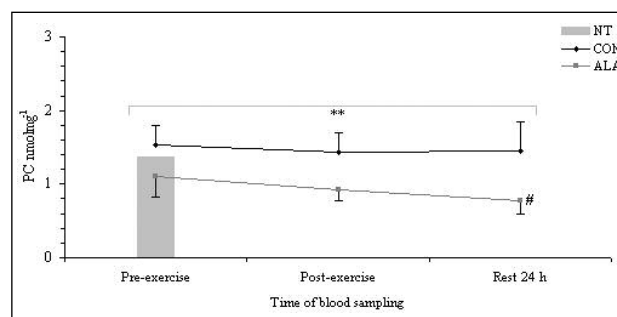


Fig. 6. Changes in plasma protein carbonylation (PC) concentration; \*\*  $P < 0.01$  indicates  $T_{\text{CON}}$  vs.  $T_{\text{ALA}}$ ; #  $P < 0.05$  indicates post-exercise vs. pre-exercise values.

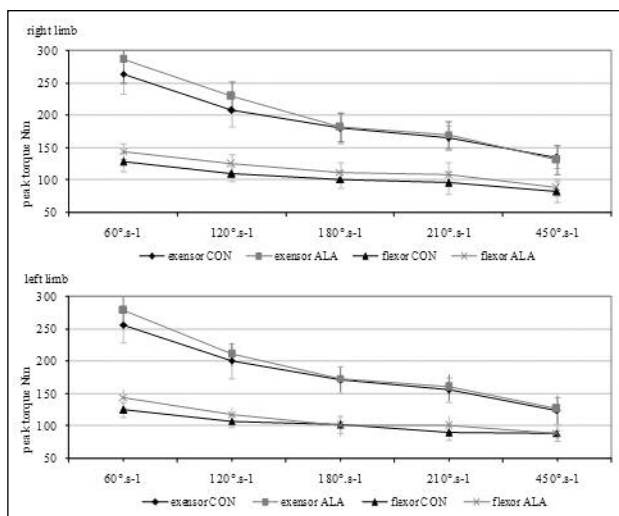


Fig. 7. Changes in total work [J] during isokinetic trial in  $T_{CON}$  (black marks) and  $T_{ALA}$  (grey marks) groups.

Exercise parameters measured by Biodex system i.e. peak torque, time to reach peak torque, total work, average power and max average peak torque were not statistically changed in subjects after ALA supplementation. However, it was observed tendency to increase in measured parameters at lower angular velocities in isometric trial e.g. the mean total work was by 5%-17% higher in  $T_{ALA}$  than  $T_{CON}$  group at angular velocity of  $60^{\circ}\cdot s^{-1}$  (Fig. 7).

## DISCUSSION

Plasma total thiols have been an integral and important part of antioxidant mechanism which regulates RONS production. The thiol level has to depend on concentration of sulphur containing compounds such as glutathione,  $\alpha$ -lipoic acid, cysteinylglycine, homocysteine and cysteine releasing from liver and muscle. The present study has shown that the level of TT in trained subjects was two-fold lower than in sedentaries. Training, particularly the resistance training, can induce a decline in plasma TT due to the high absorption of thiol compounds by active muscles (16-18). Eight-day ALA supplementation elevated the resting plasma TT concentration by 35% but did not eliminate the differences between trained and untrained subjects. This confirms that plasma TT level is determined by different sulphur-containing compounds, not only by  $\alpha$ -lipoic acid. Even though the isometric/isokinetic exercise induced muscle damage, it did not provoke any changes in plasma TT concentration in  $T_{CON}$  and  $T_{ALA}$  groups.

Our study has shown that resistance training enhanced the glutathione antioxidant system. The erythrocyte level of reduced glutathione and glutathione-related enzymes was markedly higher in trained than untrained subjects. The previous studies have demonstrated contradicting changes in GSH concentration and GR and GPx activities after physical training. For example, Kretschmar *et al.* (19) observed the higher level GSH in runners compared with sedentaries whereas Balakrishnan *et al.* (20) found the low concentration of GSH and low activity of GPx in athletes (soccers, hockey players, runners). Even though ALA has been pro-glutathione supplement, its administration did not influence the resting GSH concentration in trained subjects. However, the application of isometric/isokinetic exercise revealed the ALA action and led to changes in erythrocyte GSH, GR and GPx that were found at 24 h after exercise. In trained group, eight-day oral

ALA supplementation elevated the GSH concentration by 40% compared with  $T_{CON}$  group. It may suggest that  $\alpha$ -lipoic acid have been used only when the stress factor occurred i.e. physical exercise. The similar results were previously observed by Khanna *et al.* (4). The authors found the high level of blood and liver GSH after intragastric  $\alpha$ -lipoic acid supplementation and exhaustive treadmill exercise in rats. Busse *et al.* (21) observed in mouse cell lines that GSH content depends on dose of  $\alpha$ -lipoic acid. According Han *et al.* (22) and Moimi *et al.* (1) the high level of GSH in response to ALA supplementation has been associated with the reduction of disulfide glutathione and de novo synthesis of glutathione by improving cysteine transport.

In our study, increase in blood GSH concentration also resulted in activities of glutathione-related enzymes during recovery. The kinetic studies have demonstrated that the elevation of GSH can affect the activity of GR and GPx (23, 24).

The study has demonstrated two markers of oxidative damage, the thiobarbituric acid reactive substances (TBARS) and carbonyl groups (PC). Although the plasma TBARS and PC have been non-specific techniques, using them can offer an empirical view on the complex process of lipid peroxidation and protein carbonylation. The enhancement of peroxidation and carbonylation, followed by training or single exercise, was observed by many authors (25-28). However, our study failed to show the significant differences between trained and untrained subjects, and no changes in TBARS and PC after isometric/isokinetic exercise. We conclude that intensity of the applied isometric/isokinetic exercise was not enough to promote oxidative damage. Yet, ALA administration caused significant decrease in TBARS and PC in trained subjects. It has been very interesting that  $\alpha$ -lipoic acid maintained its antioxidant action for 48 h after the last dose of ALA.

The recent studies have proved that thiol compounds can induce some ergogenic effects. Reid *et al.* (29) were the first who have shown that thiols improved muscle performance. Then, Medved *et al.* (30) and Matuszczak *et al.* (31) have revealed that cysteine derivatives application during incremental or isometric exercises delayed muscle fatigue.

The present study has not confirmed an ergogenic action of ALA in athletes performed isometric/isokinetic exercise. The applied dose of ALA ( $600\text{ mg}\cdot\text{d}^{-1}$  for 8 days) has shown a tendency to elevate the level of parameters measured at lower angular velocities by Biodex system. It is likely that higher value of ALA could induce the significant ergogenic effects. However, a high content of cell  $\alpha$ -lipoic acid has been related with a risk of pro-oxidant reaction (2). According to Cakatay (2) a high dose of ALA could cause the removing of iron from the ferritin and stimulate the auto-oxidation of thiols. This reaction has been additionally enhanced by ascorbate with ensuing RONS production. Therefore, we have stated that long-term or high-dose application of ALA should not be recommended for athletes until the full explanation of the pro-oxidant role of  $\alpha$ -lipoic acid.

In summary, the present investigation has shown that resistance training induced a significant increase in the resting level in glutathione antioxidant system and decrease in total thiols, and no change in lipid peroxidation and protein carbonylation. The eight-day administration with  $600\text{ mg}$   $\alpha$ -lipoic acid reduced resting and post-exercise level of oxidative damage but revealed the significant effect on antioxidant glutathione system only after isometric/isokinetic exercise.

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