Free Radical Research, August 2009; 43(8): 697–705

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α-Lipoic acid modulates thiol antioxidant defences and attenuates exercise-induced oxidative stress in standardbred trotters

SUSANNA KINNUNEN[1], NIKU OKSALA[1,2], SEPPO HYYPPÄ[3], CHANDAN K. SEN[4], ZSOLT RADAK[5], DAVID E. LAAKSONEN[1,6], BERNADETT SZABÓ[7], JUDIT JAKUS[7], & MUSTAFA ATALAY[1]

[1]*Institute of Biomedicine, Physiology, University of Kuopio, PO Box 1627, Kuopio, Finland,* [2]*Division of Vascular Surgery, Department of Surgery, Tampere University Hospital, Tampere, Finland,* [3]*Equine Research, MTT Agrifood Finland, Ypäjä, Finland,* [4]*Laboratory of Molecular Medicine, Department of Surgery, Dorothy M. Davis Heart & Lung Research Institute, Ohio State University Medical Center, Columbus, OH, USA,* [5]*Faculty of Physical Education and Sport Sciences, Semmelweis University, Budapest, Hungary,* [6]*Institute of Clinical Medicine, Internal Medicine, Kuopio University Hospital, Kuopio, Finland, and* [7]*Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary*

(Received 13 March 2009; revised 28 April 2009)

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Abstract

Several micronutrient supplementation strategies are used to cope with oxidative stress, although their benefits have recently been questioned. The aim of the present study was to examine the effects of DL-α-lipoic acid (LA) in response to acute exercise and during recovery in horses. Six standardbred trotters were tested on the treadmill before and after 5-week LA supplementation (25 mg/kg body weight/day). According to electron paramagnetic resonance measurements, strenuous aerobic exercise increased significantly free radical formation in the gluteus medius muscle, which was prevented by LA supplementation. The activities of thioredoxin reductase and glutathione reductase in muscle were significantly increased in LA-treated horses, but neither LA nor exercise affected muscle thioredoxin activity. LA increased the concentration of total glutathione in muscle at rest and during recovery. Treatment with LA blunted the exercise-induced increase in plasma oxygen radical absorbance capacity and decreased the post-exercise levels of lipid hydroperoxides in plasma and malondialdehyde in plasma and in muscle. These findings suggest that LA enhances thiol antioxidant defences and decreases exercise-induced oxidative stress in skeletal muscle.

Keywords: *Thiols, redox balance, lipid peroxidation, exercise, horse*

Introduction

Acute physical exercise generates reactive oxygen species (ROS) in skeletal muscle, which may result in oxidative stress and oxidative modification of molecules [1]. However, ROS also modulate gene expression via redox-sensitive transcription pathways and represent an important cellular regulatory mechanism. The detection of reactive species generally relies on indirect measurements due to their short lifetime. Electron paramagnetic resonance (EPR) spectroscopy, with or without spin traps, may provide highly sensitive measurements of reactive free radicals [2–5].

The contemporary definition of oxidative stress is the disruption of thiol redox circuits leading to imbalance in cell signalling and dysfunctional redox-control. Thus, the function and homeostasis of thiol pathways are central characteristics of redox control [6,7]. Moreover, the adaptation of the endogenous antioxidant circuits in response to regular physical activity is a potential mechanism to enhance tolerance of skeletal muscle to exercise-induced stress and trigger other important physiological adaptations [8].

Correspondence: Mustafa Atalay, MD, PhD, Institute of Biomedicine, Physiology, University of Kuopio, PO Box 1627, Kuopio, Finland. Tel: +358 400 173972. Fax: +358 17 162889. Email: mustafa.atalay@uku.fi

ISSN 1071-5762 print/ISSN 1029-2470 online © 2009 Informa UK Ltd.
DOI: 10.1080/10715760903037673

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Dietary micronutrient supplementations have been reported to increase the total antioxidant status and to enhance the cellular protection against exercise-induced oxidative stress and muscle damage. R-α-lipoic acid (RLA) is a natural thiol compound present in bound form in all animal cells, but is usually administered as a racemic mixture. It is considered a metabolic antioxidant and a redox-modulator that decreases exercise-induced oxidative stress and at the same time supports cellular metabolic processes [9]. α-Lipoic acid (LA) is both water- and lipid-soluble and therefore is able to penetrate cell membranes and exert multiple effects on the cell. It is also capable of regenerating reduced endogenous antioxidants like glutathione and vitamin C [9]. Within cells, LA modulates ion transport [9] and plasmamembrane redox system [10]. LA has also been reported to protect cells against the detrimental effects of high levels of hydrogen peroxide (H_2O_2), subsequent reversible protein modifications and transient enzyme inhibition [11].

On the other hand, antioxidant thiol compounds, including LA, may have pro-oxidant properties depending on the type of stress or physiological circumstance [12–14]. The efficacy of non-protein bound LA to function as a physiological antioxidant has been recently questioned [15]. Here, we hypothesize that the beneficial antioxidant and cell supportive characteristics of LA outweigh its potential pro-oxidant actions. We aimed to clarify the effects of 5-week LA supplementation on the formation of free radicals in equine muscle after strenuous treadmill exercise using EPR measurement. We used the horse, a good animal model to study oxidative stress because of its high maximal oxygen uptake (VO_{2max}), which makes it vulnerable to exercise-induced oxidative insults. We also studied the effects of LA on diverse oxidative stress markers and antioxidant responses at rest, immediately after acute exercise and during recovery, because the effects of LA on tissue thiol antioxidant network in relation to acute exercise were not clear until now.

Material and methods

Animals, exercise protocols and supplementation

The experimental protocol was approved by the Ethics Committee of the MTT Agrifood Research Finland. Six clinically healthy standardbred trotters, 5–13 years of age and 400–508 kg in weight, were examined in this study. Two of the horses were mares and four were geldings. All horses had been in regular training for several years. The horses were housed in box stalls and fed hay silage (*ad libitum*) and oats (2.2 ± 0.24 kg) to meet the recommended nutrient requirements [16] and to maintain a moderate body condition score [17].

Before starting this series of tests, the administration of additional vitamins was discontinued for 5 weeks (control period) to rule out a previous supplementation effect. The performance tests were carried out before and after LA supplementation. Prior to each performance test, the individual treadmill speed (V_{La4}) resulting in a blood lactate level of 4 mmol/l was determined for each horse with the standardized exercise test (SET). The SET consisted of a 10-min warm-up period at 1.7 m/s, followed by four trotting intervals, 2 min each, at speeds of 7, 8, 9 and 10 m/s on a high-speed treadmill with a 2.5° incline. Blood samples for lactate analysis were collected before the test and during the last 10 s of each interval. Exercise speed causing a blood lactate level of 4 mmol/l (V_{La4}) was calculated from the velocity of the treadmill and blood lactate concentration in the SET [18]. In the subsequent performance test the treadmill speed was kept under the anaerobic threshold, i.e. under the individual V_{La4} to ensure that lactic acid will not accumulate in the skeletal muscles. The performance test protocol is presented in Table I. The results of the first performance test prior to the LA supplementation are further considered as control.

After a 5-week control period, DL-LA (Changshu Fushilai Medicine & Chemical Co., Ltd, China) mixed in molasses was supplemented to the horses at 25 mg/kg body weight/day for five consecutive weeks. The purity of LA was confirmed by comparing with reagent grade LA using HPLC [19].

Samples

Blood samples were drawn from the jugular vein at rest and immediately after exercise and at 2, 6, 24 and 48 h of the recovery after control and LA supplementation periods. The samples were collected in lithium-heparin tubes and centrifuged immediately to separate plasma for biochemical analysis. Plasma

Table I. Procedure for the performance tests and sampling times.

Treadmill speed ($m^{-1}\ s^{-1}$)	Time (min)	Gait
Blood and muscle samples (rest)		
1.7	15	walk (warming up)
6.2–6.8	10	trot
1.7	10	walk
6.2–6.8	10	trot
1.7	10	walk
6.2–6.8	10	trot
Blood samples (post-ex)		
1.7	10	walk
Active cooling down (10 min)		
Blood samples (after 2 h recovery)		
Blood and muscle samples (after 6 h recovery)		
Blood and muscle samples (after 24 h recovery)		
Blood and muscle samples (after 48 h recovery)		

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samples were aliquoted and snap-frozen in liquid nitrogen and kept at –80°C until analysed.

For practical reasons we were forced to limit the number of muscle samples. Tissue samples from the middle gluteal muscle were obtained at rest and after 6, 24 and 48 h of recovery. In addition, muscle biopsies for EPR and TGSH/GSSG-analysis were taken immediately after the last interval (later referred as post-exercise), before the active cooling period. Biopsy specimens were obtained under local anaesthesia as described previously [20]. The samples were first rinsed quickly with ice-cold saline solution and blotted onto filter paper, then snap frozen in liquid nitrogen for further analysis. The muscle samples were snap frozen in a steel funnel to form a cylinder-like shape and stored in liquid nitrogen until analysis.

For other assays from muscle, the frozen tissue samples were ground in liquid nitrogen and homogenized in 0.1 M phosphate buffer, pH 7.4, containing a protease inhibitor cocktail. Muscle homogenates were stored at –80°C until analysis. Unless otherwise stated, all chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and were of analytical grade or the highest grade available.

Analyses

EPR spectroscopy is a direct method for detection of free radicals and was used to measure the *in vivo* generated steady-state free radical concentration of muscle samples. Measurements were performed in a quartz finger-Dewar filled up with liquid nitrogen as described earlier [4].

Thioredoxin (TRx) activity was determined using an endpoint assay (IMCO Corporation Ltd AB, Sweden), the principle of the assay being the rapid reaction between reduced thioredoxin and protein disulphide. The thioredoxin reductase (TRxRd) assay (IMCO) was based on the same principals using a relative excess of thioredoxin.

Oxygen radical absorbing capacity (ORAC) assays were performed using a multi-well plate reader as previously described [21]. The antioxidant capacity of the samples was measured by the inhibition of the decrease of the fluorescence of fluorescein (FL, Na salt, Riedel-De Haen Aldrich Milwaukee, WI). Trolox (Aldrich, Milwaukee, WI) was used as a control standard. Final results were calculated using the differences of areas under the FL decay curves between the blank and a sample and quantified according to Trolox standards and expressed as μmol.

Lipid hydroperoxides (LPO) in whole plasma were determined as described by Arab and Steghens [22], which is based on oxidation of Fe II to Fe III by lipid hydroperoxides under acidic conditions, followed by complexation of Fe III by xylenol orange. Peroxidative damage to cellular lipid constituents was determined by measuring the total malondialdehyde (MDA) in plasma and muscle and was measured according to the method of Gerard-Monnier et al. [23].

The activities of muscle glutathione peroxidase (GPx) and glutathione reductase (GRd), total glutathione concentration (TGSH) and the concentrations of oxidized glutathione (GSSG) in muscle were determined spectrophotometrically as described previously [24]. The tissues were deproteinized with metaphosphorous acid (MPA) for TGSH and GSSG analysis.

Protein carbonyls were measured as a marker of protein oxidation. Oxidized proteins were derived by 2-4-dinitrophenylhydrazine (DNPH) and measured using Western blot [25] for muscle homogenates and ELISA for plasma samples [26].

Statistical analyses

Data were analysed using SPSS for Windows version 14.0. A multivariate linear mixed model was used to assess whether duration of exercise and use of LA have an effect on physical quantities, as it takes into account the correlation structure of the data due to repetitions. Antioxidants and oxidative stress-related parameters (TRx, TRxRd, TGSH, GSSG, GRd, GPx, ORAC, protein carbonyls in plasma and muscle, LPO and MDA in plasma and in muscle) were used as dependent variables and LA supplementation (on/off), as well as sampling points (at rest, post-exercise, 2-, 6-, 24- and 48-h of recovery) were considered as fixed effect factors and an individual horse as a random effect factor. There were no covariates used. Also the paired samples *t*-test was used to assess whether the use of lipoate has any effect on muscle free radical production (EPR signal) before and after exercise. Spearman's correlation coefficient was used to measure correlation between variables. *p*-values less than 0.05 were treated as statistically significant.

Results

Based on EPR measurements, LA supplementation blunted the exercise-induced free radical accumulation in skeletal muscle. The EPR signal intensity increased from rest to immediately after exercise in control group ($p < 0.01$, Figure 1a and b), whereas no change was seen in the LA-supplemented group. However, there were no statistically significant difference between the LA-supplemented and control groups after exercise ($p = 0.297$).

There was no significant change in muscle TRx activity following exercise or LA supplementation (Figure 2a). LA supplementation had a main increasing effect for TRxRd activity ($p < 0.05$, Figure 2b).

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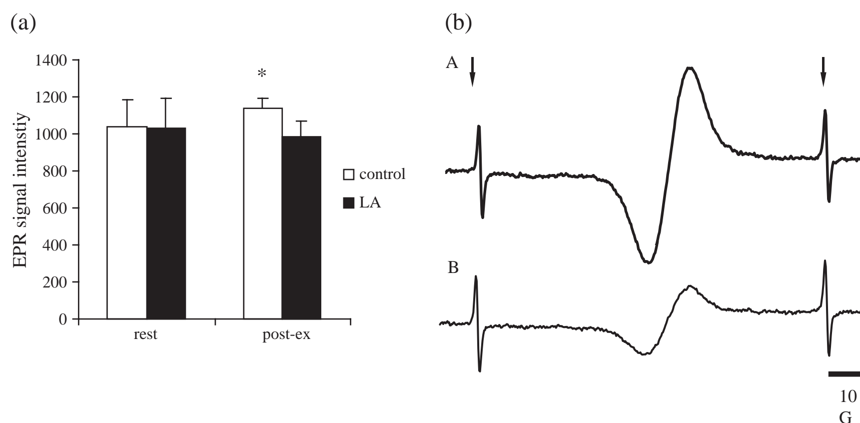

Figure 1. Effect of exercise and LA treatment on the amount of free radicals in the muscle of the horse. (a) The values are means ± SEM, bar marked with * differs significantly ($p < 0.05$) from its counterpart at rest. (b) EPR spectra taken *ex vivo* at 77 K showing steady-state native free radical concentration of LA supplemented horses muscle tissue taken before (A) and after (B) exercise. Arrows on the spectrum indicate the signal of Mn/MnO internal standard.

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There was a negative correlation between TRxRd at rest and post-exercise amount of free radicals following LA supplementation (Table II). TRx activity correlated positively with the amount of TGSH in skeletal muscle during 24- and 48-h recovery period in both non-supplemented and LA-supplemented horses (Table II). In addition, in the LA-supplemented group at 48-h recovery, muscle TRx activity was negatively correlated with plasma AST levels (Table II).

There was also a main increasing effect of LA supplementation for muscle TGSH levels ($p < 0.05$, Figure 3a) and after 6-h recovery, TGSH levels were significantly higher in LA-supplemented horses than in non-supplemented horses ($p < 0.05$). There were no significant changes with exercise or during recovery in muscle GSSG or the glutathione redox ratio (GRR% = GSSG/TGSH × 100) in non-supplemented or LA-supplemented horses (Figure 3b and c). However, the post-exercise free radical amount correlated positively with post-exercise GSSG concentration in muscle following LA supplementation (Table II). Muscle GRd activity was significantly higher in LA-supplemented horses than in non-supplemented animals after 24-h recovery (Figure 4a). There was also a main increasing effect of LA supplementation for muscle GPx activity ($p < 0.01$, Figure 4b).

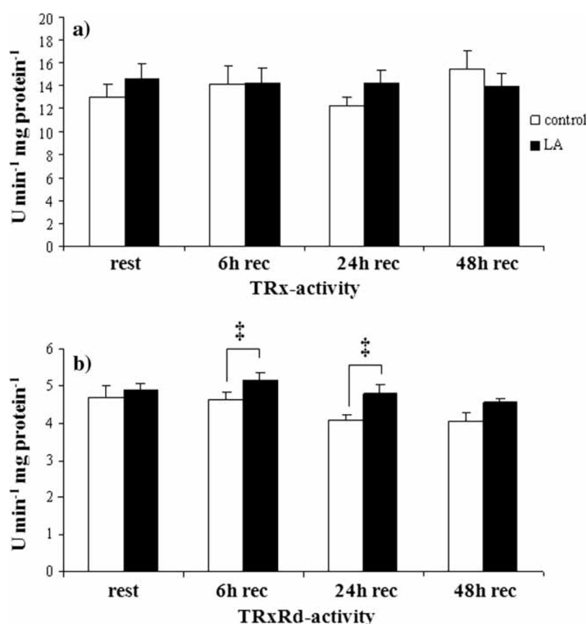

Figure 2. Effect of exercise and LA treatment on (a) TRx-activity and (b) TRxRd-activity in the muscle of the horse. Values are means ± SEM, bars marked with ‡ differ significantly ($p < 0.05$) between treatments.

In non-supplemented horses, intense acute exercise increased the plasma ORAC ($p < 0.01$, Table III) immediately after the exercise. ORAC also remained higher after 2 h of recovery ($p < 0.05$). LA supplementation attenuated the overall exercise-induced ORAC response compared with controls ($p < 0.05$).

LA supplementation had no statistically significant effect on the muscle protein carbonyl levels (Table III). Five-week LA administration decreased the exercise-induced LPO in plasma compared with control ($p < 0.05$, Table III). Moreover, LA supplementation had a main blunting effect for exercise-induced plasma and muscle MDA concentrations ($p < 0.05$ in both, Table III). The overall trend in plasma and muscle MDA levels was consistent with those of plasma and muscle LPO.

Discussion

In the present study, we observed that 5-week LA supplementation decreased free radical formation as measured by EPR spectroscopy in response to strenuous aerobic exercise in horse skeletal muscle. This finding supports the earlier reports that both LA and its reduced form DHLA directly scavenge ROS [15]. In addition, 5-week LA supplementation

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Table II. Significant correlations between different biochemical variables.

	Control/LA	r	P
Resting TRxRd vs Post-ex EPR	LA	−0.900	<0.05
Post-ex TGSH vs Post-ex EPR	Control	0.900	<0.05
Post-ex GSSG vs Post-ex EPR	LA	1.000	<0.001
TRx 48-h rec vs TGSH 48-h rec	Control	0.900	<0.05
TRx 24-h rec vs TGSH 24-h rec	LA	0.900	<0.05
TRx 48-h rec vs TGSH 48-h rec	Control	0.980	<0.01
TRx 48-h rec vs TGSH 48-h rec	LA	0.980	<0.01
TRx 48-h rec vs AST 48-h rec	LA	−0.975	<0.01
Muscle PCarb 48-h rec vs TRxRd 48-h rec	Control	0.900	<0.05
Muscle PCarb 24-h rec vs TRxRd 24-h rec	LA	0.900	<0.05
Muscle PCarb 24-h rec vs TRx 24-h rec	LA	0.900	<0.05
Plasma PCarb 24-h rec vs TRxRd 24-h rec	LA	0.900	<0.05
Plasma PCarb 6-h rec vs Resting TRx	Control	−0.900	<0.05
Plasma PCarb 24-h rec vs Resting TRx	Control	−0.900	<0.05
Plasma PCarb 24-h rec vs TRx 24-h rec	LA	0.900	<0.05
Plasma PCarb 48-h rec vs TRx 48-h rec	LA	−0.975	<0.01
Resting GSSG vs Resting TRx	Control	0.900	<0.05
Resting GRR vs Resting TRx	Control	0.900	<0.05
TGSH 24-h rec vs Plasma ORAC 24-h rec	LA	0.900	<0.05
Post-ex EPR vs Plasma LPO 24-h rec	Control	0.900	<0.05
Resting TRxRd vs Muscle LPO 24-h rec	LA	−0.900	<0.05
Resting TRxRd vs Muscle MDA 24-h rec	LA	−0.900	<0.05
CK 6-h rec vs Muscle LPO 6-h rec	Control	0.829	<0.05
CK 24-h rec vs Muscle LPO 24-h rec	Control	0.829	<0.05
AST 24-h re vs Plasma MDA 24-h rec	Control	−1.000	<0.001
AST 6-h rec vs Muscle LPO 6-h rec	LA	−0.900	<0.05

Abbreviations: muscle thioredoxin (TRx) and thioredoxin reductase (TRxRd), electron paramagnetic resonance (EPR), concentrations of muscle total glutathione (TGSH) and oxidized glutathione (GSSG), glutathione redox ratio (GRR), plasma aspartate aminotransferase (AST) and creatine kinase (CK), protein carbonyl (PCarb) concentrations in plasma and muscle, oxygen radical absorbance capacity (ORAC) of plasma and lipid hydroperoxides (LPO) and malondialdehyde (MDA) in plasma and muscle.

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up-regulated muscle TGSH levels and increased the activities of TRxRd and GRd in muscle during recovery, but had no effect on muscle TRx activity.

TRx and TRxRd play an essential role in cell function and protection by limiting oxidative stress directly via their antioxidant effects and indirectly by protein–protein interactions with key signalling molecules [27]. The protective role of TRx system against oxidative insults is further supported in the present study by the negative correlations between TRxRd activity at rest and post-exercise free radical formation. In addition, we observed positive correlations between muscle protein carbonyl levels and TRx and TRxRd activities after 24-h recovery in LA-supplemented horses. Induction of antioxidant defences to acute stress can be interpreted as a protective response and the extent of this induction may reflect the levels of oxidative stress [9]. Other studies have reported decreases in the levels of protein carbonyls after LA supplementation in response to exercise training, but these studies did not examine acute responses to exercise-induced oxidative stress [28,29].

We found no significant changes in GSSG or the GSSG-TGSH ratio in response to exercise or during recovery. The glutathione redox ratio is considered to be an indicator of tissue redox status [30] and oxidative stress [7]. The changes in redox ratios occurring with certain types of exercise have not always been consistent among studies, even when using similar protocols [31–34]. This holds true with horses too [21,35–43]. Changes in glutathione status are short-lived, likely due to rapid conversion of GSSG back to GSH by GRd, which is an important determinant of GRR and cellular protection against oxidative stress [31,44].

LA supplementation increased GRd activity after 24-h recovery, suggesting increased protection against exercise-induced oxidative damage. It has been reported previously that 8-week LA supplementation combined with treadmill training and exhaustive exercise had no effect on muscle GRd activity immediately after one bout of acute exercise until exhaustion [45]. On the other hand, supplementation of LA together with carnitine increased the activity of GRd in skeletal muscle in the resting state in aged rats [46].

In the present study, the increase in TGSH concentration in muscle during recovery can be attributed to the enhanced regeneration of GSH through increased GRd-activity and to the GSH-sparing effect of LA [45,47]. In LA-supplemented horses higher TRx activity at rest was associated with lower post-exercise GRR%, supporting the hypothesis of TRx protection against oxidative stress. It has been reported earlier that LA had no effect on GSH

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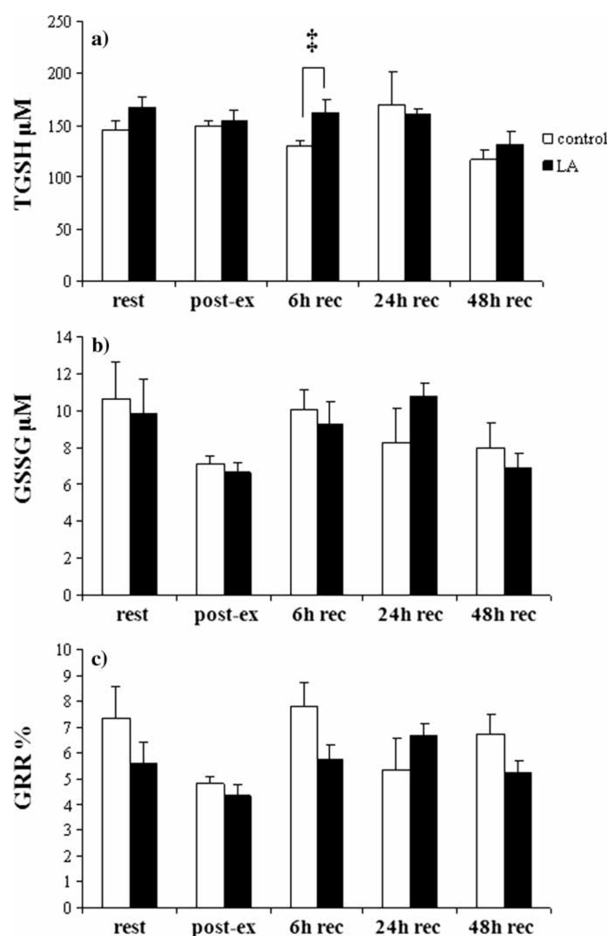

Figure 3. Effect of exercise and LA treatment on (a) TGSH, (b) GSSG and (c) GRR (%) in muscle of the horse. Values are means ± SEM, bars marked with ‡ differ significantly ($p < 0.05$) between treatments.

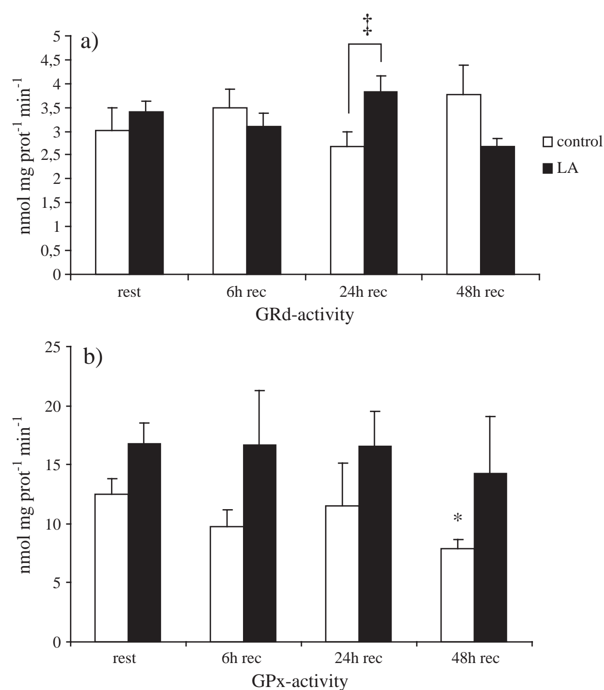

Figure 4. Effect of exercise and LA treatment on (a) GRd-activity and (b) GPx-activity in the muscle of the horse. Values are means ± SEM, bars marked with ‡ differ significantly ($p < 0.05$) between treatments and bar marked with * differs significantly ($p < 0.05$) from its counterpart at rest.

concentration in red or white blood cells [48]. On the other hand, our group has previously reported that LA supplementation increased total glutathione levels in liver and blood [45].

There was an overall tendency for muscle GPx activity to increase after LA supplementation in the present study, which is consistent with the previous studies [46,49]. In horses GPx activity has increased in erythrocytes following antioxidant supplementation with daily quantities of 11 500 mg ascorbic acid, 7000 mg α-tocopherol acetate, 500 mg β-carotene and the following trace elements: Cu 187 mg, Zn 769 mg and Se 7 mg [50] and in white blood cells following LA supplementation [48].

The immediate increase in the ORAC after acute exercise in non-supplemented horses is in accordance with our previous study with standardbred trotters [21] and with other studies in horses where plasma antioxidant capacity (PAOC) and total antioxidant reactivity (TAR) methods were used [51]. The exercise-induced increases in plasma antioxidant protection have been suggested to be a result of haemo-concentration [51,52]. However, this assumption may not explain our results, because LA supplementation blunted the exercise-induced increase in ORAC. Our observation is also supported by White et al. [51], who showed that intravenous ascorbate administration to horses increased the PAOC and TAR under basal conditions, with no increase in response to exercise. It has been reported that micronutrient supplementations *per se* up-regulate total antioxidant capacity of rats [53] and horses [54]. It is therefore possible that the antioxidant properties of LA may preserve the antioxidative capacity of the horse during exercise and attenuate the amount of acute oxidative stress, decreasing the need for an exercise-induced increase of endogenous antioxidants [12].

Because ORAC may better reflect water-soluble antioxidant status [55], the increase in ORAC may therefore have minor effects on lipid-phase oxidant status. LA exerts its antioxidant effects both in water and lipid phase. In the present study, the protective effects of LA supplementation in the lipid phase were more evident as represented by decreased oxidative lipid damage. This is supported by a previous study by our group [45] and by more recent findings in horses [48,56], rats [53] and humans [57]. The decrease in lipid peroxidation markers and retained fluidity of cell membranes is further supported by our recent observations, where exercise induced the activity of creatine kinase (CK) and AST to a lesser extent in LA-supplemented horses than in non-supplemented horses (Kinnunen et al., unpublished

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Table III. Effects of α-lipoic acid (LA) on oxygen radical absorbing capacity (ORAC) and the concentrations of lipid hydroperoxides (LPO), malondialdehyde (MDA) and protein carbonyls (PCarb) in plasma and in middle gluteal muscle of the horse at rest, immediately after intense aerobic exercise and during recovery.

				Recovery			
		At rest	Post-exercise	2 h	6 h	24 h	48 h
Plasma							
ORAC (μmol/L)	con	35.7±2.03	43.2±1.40*	43.5±1.23*	34.3±1.43	33.0±2.03	34.4±1.69
	LA	33.4±3.28	35.3±1.68‡	34.7±3.11‡	33.8±5.45	33.5±1.56	32.8±2.37
LPO (μM)	con	7.0±1.26	12.9±2.63*	8.0±1.46	7.1±1.98	5.7±2.09	3.6±1.43
	LA	5.9±0.95	8.7±1.82‡	5.8±0.95‡	3.3±1.31	5.6±0.88	2.7±0.61
MDA (μM)	con	2.1±0.09	3.1±0.67*	2.7±0.57	2.4±0.28	2.4±0.69	1.8±0.22
	LA	2.1±0.23	2.6±0.15	2.2±0.20	2.0±0.15	1.9±0.14	1.7±0.16
PCarb (nmol/mg protein)	con	0.99±0.050	1.02±0.035	0.98±0.063	1.00±0.026	0.96±0.035	1.01±0.061
	LA	1.05±0.880	1.03±0.056	1.00±0.065	1.04±0.042	1.00±0.045	1.03±0.030
Muscle							
LPO (μM)	con	134.9±15.37	N.A.	N.A.	108.3±6.43*	103.1±3.45*	99.1±7.02
	LA	110.2±3.00‡	N.A.	N.A.	105.3±6.87	108.6±3.72	107.2±4.16
MDA (μM)	con	3.7±0.54	N.A.	N.A.	3.3±0.64	2.8±0.14	2.5±0.21*
	LA	2.7±0.30‡	N.A.	N.A.	2.9±0.38	2.3±0.17	2.2±0.11
PCarb (Arbitrary units)	con	1.0±0.08	N.A.	N.A.	1.4±0.19*	1.1±0.09	0.9± 0.06
	LA	1.2±0.07	N.A.	N.A.	1.2±0.03	1.0±0.13	0.9±0.09

Values are means ± SEM, level of significance was set at $p < 0.05$. Values marked with * differ significantly from the resting counterpart and on LA-treatment row the values marked with ‡ differ significantly from the non-treated horses at the same time point. N.A. (not available).

observations). Concentrations of muscle LPO and plasma MDA were strongly associated with the plasma activities of CK and AST, suggesting a strong association between lipid peroxidation and muscle damage. These findings are supported by the earlier studies in young and older men [58].

In summary, we show that LA supplementation controlled strenuous aerobic exercise-induced oxidative insult in skeletal muscle, improved glutathione redox status and enhanced thioredoxin reducing capacity. These results give new insight on the role of thiol antioxidants to decrease the risks of strenuous physical exercise. The expression of antioxidant enzymes and up-regulation of other defence mechanisms appears to be induced by ROS generated during exercise, suggesting that the generation of ROS is an essential signal in the adaptation to exercise [2,8,59] and the adaptive responses of skeletal muscle to unaccustomed contractions [2,12]. It has been suggested nearly two decades ago that for maximal performance an optimal level of ROS is needed [60]. Therefore, the over-supplementation of nutritional antioxidants may decrease the maximal performance by attenuating essential ROS production.

Our results suggest that LA-supplementation may reduce the indices of exercise-induced oxidative stress directly by augmenting intracellular protective mechanisms against oxidative insult or indirectly by decreasing ROS production during exercise. Nonetheless, one should still bear in mind that, although supplementation with particular micronutrients decreases exercise-induced oxidative stress, there may also be a risk of attenuating the normal physiological response of tissues to exercise and blunting the training-induced adaptations.

Acknowledgements

The skillful technical assistance of Ms Taina Vihavainen, Ms Taija Hukkanen and Ms Satu Marttila is gratefully acknowledged. The authors would like to thank David Alan Carlson, PhD, for valuable suggestions, DVM Kristiina Palttala, Ms Marjatta Lehtisaari and MSc Marja-Leena Hannila for their valuable help and support during the study; and the staff and the students of Ylä-Savo Vocational School for the facilities.

The study has been supported by the grants from Erkki Rajakoski Foundation, Helsinki to S.K., the Finnish Ministry of Education and COST actions B35 and BM0602.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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