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Effects of supplementation with α-lipoic acid on exercise-induced activation of coagulation

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Abstract

Physical exercise leads to minor activation of blood coagulation, which appears to be balanced by a concomitant activation of the fibrinolytic system. The mechanisms underlying this physiological phenomenon are still unknown. To evaluate the role of oxidative stress for exercise-induced activation of coagulation, we investigated if supplementation with α-lipoic acid (LA) as an antioxidant reduces the hemostatic response to exercise. Ten young men (age, 25 ± 4 years; maximal oxygen consumption [$\dot{V}O_2$max], 61 ± 6 mL/(kg min) [mean ± SD]) were subjected to a 1-hour run on a treadmill at a velocity corresponding to an oxygen demand of 75% to 80% of maximum (anaerobic threshold). Exercise testing was repeated in the same subjects after supplementation with LA (1200 mg/d PO) for 10 days. Molecular markers of thrombin (prothrombin fragment 1 + 2, thrombin-antithrombin complexes) and fibrin formation (fibrinopeptide A) as well as markers of the fibrinolytic activity (tissue-plasminogen activator, plasmin-antiplasmin complexes, D-dimers) and of lipid peroxidation (malondialdehyde) were determined before and immediately after exercise. Supplementation therapy with LA had no effect on hemostatic and fibrinolytic variables either at rest or in response to exercise. Likewise, concentrations of malondialdehyde at rest and after exercise were not influenced by LA. In summary, the hemostatic response to exercise is not affected by supplementation with LA in young healthy male individuals. The role of oxidative stress for exercise-induced activation of coagulation has to be defined in further studies.

1. Introduction

In healthy individuals, physical exercise leads to an activation of the coagulation system, which may result in minor formation of thrombin and fibrin when exercise is prolonged and exhaustive [1-3]. The mechanisms by which physical exercise mediates hemostatic activation are still unknown. Factors that are probably relevant include the great increase of shear stress and the release of catecholamines, particularly with respect to activation of platelets with exercise [4,5]. Given that intensive physical exercise leads to the generation of reactive oxygen species [6,7], which are involved in the regulation of coagulation (eg, expression of tissue factor is regulated by the redox-sensitive transcription nuclear factor κB [NF-κB] [8]), we hypothesized that oxidative stress might play a role for exercise-induced activation of coagulation. To test this hypothesis, we investigated whether the hemostatic response to exercise can be influenced by supplementation with α-lipoic acid (LA) as a natural antioxidant. Therefore, 10 healthy individuals were subjected to an exercise model that has been shown to induce formation of thrombin and fibrin [2] (ie, a 1-hour run on a treadmill at a velocity corresponding to an oxygen demand of 75% to 80% of maximum [anaerobic threshold]). Molecular markers of thrombin (prothrombin fragment [PTF] 1 + 2, thrombin-antithrombin complexes) and fibrin formation (fibrinopeptide A [FPA]) as well as markers of the fibrinolytic activity (tissue plasminogen activator [tPA], plasmin-antiplasmin complexes, D-dimers) were determined before and after exercise testing with and without supplementation with LA (1200 mg daily for 10 days). Plasma levels of malondialdehyde (MDA) were measured as an index of oxidative stress.

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2. Subjects and methods

2.1. Subjects

Ten healthy endurance-trained young men (age, 25 ± 4 years) were studied. Physical examination, electrocardiogram, and routine laboratory parameters revealed no pathological findings. All subjects were nonsmokers and had no evidence of thromboembolic disease in their personal and family history. Participants were not allowed to take any medication (eg, aspirin, antioxidants) 2 weeks before exercise testing.

The study protocol was approved by the ethical committee for human studies of the Medical Faculty at the University of Heidelberg. All participants gave their written informed consent before entering the study.

2.2. Exercise testing

To assess individual performance status and speed at the individual anaerobic threshold, participants were subjected to an incremental graded exercise test on a treadmill (slope, 1.5%) starting at an initial velocity of 8 km/h with increments of 2 km/h every 3 minutes until subjective exhaustion. Maximal oxygen consumption ($\dot{V}O_2max$) was measured by means of a metabolic cart (Oxycongamma, Mijnhardt, Bunnik, The Netherlands) applying an open-circuit method. The individual anaerobic threshold was determined as described elsewhere [9] and corresponded to 75% to 80% of $\dot{V}O_2max$.

Within 2 weeks after determination of $\dot{V}O_2max$, subjects performed a 1-hour run on a treadmill at a standardized velocity corresponding to the anaerobic threshold. Heart rate was recorded continuously by a sport tester (Polar Electro, Kempele, Finland), and plasma lactate was measured every 15 minutes during exercise to calculate a mean plasma lactate concentration.

2.3. Supplementation with LA

The 1-hour run was repeated in the same subjects after supplementation with LA (Thioctacid 600 HR, Asta Medica, Frankfurt, Germany) for 10 days (2 × 600 mg/d PO). The last medication was taken in the presence of medical staff 30 minutes before exercise testing was started. All exercise tests were run between 9:00 AM and noon.

Anthropometric data and characteristics of exercise testing are summarized in Table 1.

Table 1
Anthropometric data and characteristics of exercise testing in 10 subjects with and without supplementation with LA

	Mean ± SD		
Age (y)	25 ± 4		
BMI (kg/m^2)	22.6 ± 1.8		
Maximal heart rate (1/min)	193 ± 7		
$\dot{V}O_2max$ [mL/(kg min)]	61.4 ± 6.0		
1-h exercise testing	Without LA	With LA	P[a]
Velocity (km/h)	13.4 ± 1.2	13.4 ± 1.2	NS
Heart rate$_{mean}$ in %heart rate$_{max}$	89 ± 4	89 ± 4	NS
Mean plasma lactate (mmol/L)	2.8 ± 0.8	2.6 ± 0.8	.059
Borg scale	14 ± 2	14 ± 2	NS
Plasma volume change (%)	−3.8 ± 7.4	−5.5 ± 5.9	NS

BMI indicates body mass index.
[a] Wilcoxon test.

2.4. Blood sampling and laboratory methods

Venous blood samples were collected before and within 3 minutes after exercise by a clean venipuncture (19-gauge needle) from an antecubital vein under controlled venous stasis of 45 mm Hg using the Sarstedt system (Sarstedt, Nümbrecht, Germany). To obtain baseline values, samples before exercise were drawn after subjects had rested for 30 minutes in a supine position.

Blood samples were collected in the following sequence:

1. A total of 4.5 mL venous blood was added to 0.5 mL of CTAD-PPACK as anticoagulant (stock solution containing 25 mL of citrate-theophylline-adenosine-dipyridamole [Becton Dickinson, Rutherford, NJ] plus 5 mg of phenyl prolyl arginine-chloromethylketone [Calbiochem, La Jolla, Calif], giving a PPACK concentration of 382 μmol/L) for measurement of PTF 1 + 2 (Enzygnost-F1 + 2, Behring, Marburg, Germany), thrombin-antithrombin complexes (Enzygnost-TAT, Behring), and FPA (radioimmuno reagents supplied by Imco, Stockholm, Sweden) as described in detail previously [10].
2. A total of 4.5 mL blood was added to 0.5 mL of 0.106 mol/L trisodium citrate for determination of activated partial thromboplastin time with use of Pathromtin (Behring), of fibrinogen according to the method of Clauss, of tissue-plasminogen activator (tPA) antigen (TintElize tPA, Biopool, Umea, Sweden), of plasmin-antiplasmin (PAP) complexes (Enzygnost PAP, Behring), and of D-dimers (Enzygnost D-Dimer micro, Behring).
3. A total of 4.5 mL blood was added to 7.5 mg dry EDTA for hemoglobin level determination (Coulter Counter, Coulter Electronics, Krefeld, Germany) and for measurement of hematocrit (microhematocrit centrifuge, Hettich, Tuttlingen, Germany) in duplicate after 6 minutes of centrifugation at 13 000g.
4. Plasma concentrations of MDA were determined from derivatized plasma samples by means of reversed-phase liquid chromatography and fluorescence detection.

Samples (1) and (2) were rapidly put onto melting crushed ice for 10 minutes and thereafter centrifuged at 4°C for 30 minutes at 2000g. Multiple aliquots of plasma were snap frozen in liquid nitrogen and stored at −80 °C until

analysis. Changes in plasma volume were calculated according to the method of Dill and Costill [11]. The results for proteins with a molecular weight greater than 30 000 [12] (ie, all except for FPA) were corrected for changes of plasma volume occurring during and after exercise by the following factor: (100 + ΔPV)/100, where ΔPV is the change of plasma volume given in percent.

2.5. Statistical analysis

Differences in exercise characteristics in subjects before and after supplementation with LA as well as effects of exercise on coagulation parameters and MDA were analyzed by nonparametric testing (Wilcoxon test). Wilcoxon tests were also applied to investigate the effects of supplementation with LA on coagulation parameters and MDA at rest and in response to exercise. For these 3 multiple comparisons in the same individuals, a Bonferroni adjustment was made multiplying a particular P value by 3.

Correlations between coagulation parameters with and without supplementation with LA and MDA were analyzed by the Pearson correlation coefficients. The level of statistical significance was set at $P < .05$. Data are given as mean ± SE unless otherwise stated.

3. Results

Except for mean plasma lactate concentrations during exercise that tended to be lower under LA (2.6 ± 0.8 nmol/L [mean ± SD] vs 2.8 ± 0.8 nmol/L; $P = .059$), supplementation with LA did not affect characteristics of exercise testing (Table 1).

3.1. Effects of LA on hemostasis

Resting values of all hemostatic variables (ie, activated partial thromboplastin time, fibrinogen, PTF 1 + 2, TAT, and FPA) were not influenced by LA (Table 1 and Figs. 1 and 2).

Maximal running exercise induced the known moderate increase of hemostatic variables, with PTF 1 + 2 rising by 0.13 ± 0.02 nmol/L ($P < .05$), TAT by 0.65 ± 0.17 ng/mL ($P < .05$), and FPA by 0.85 ± 0.19 ng/mL ($P < .05$). Supplementation with LA, however, had no effect on exercise-induced changes of hemostatic variables, with increases in plasma levels of PTF 1 + 2 by 0.12 ± 0.02 nmol/L ($P < .05$ vs baseline; NS as compared with exercise-induced changes without LA), of TAT by 0.60 ± 0.15 ng/mL ($P < .05$, NS, respectively), and of FPA by 0.87 ± 0.18 ng/mL ($P < .05$, NS, respectively).

3.2. Effects of LA on fibrinolysis

Fibrinolytic variables at rest (ie, tPA, PAP, and D-dimers) remained essentially unchanged after supplementation with LA (Table 2). In response to exercise, markers of plasmin formation increased considerably, with plasma levels of tPA and PAP complexes rising 5-fold and 4-fold, respectively ($P < .05$, $P < .05$, respectively). Maximal running exercise enhanced fibrinolytic activity as reflected by increases of D-dimers by 3.7 ± 1.1 ng/mL ($P < .05$).

Supplementation with LA, however, did not affect exercise-induced changes of fibrinolytic variables (Table 2).

3.3. Effects of LA on MDA

Exercise testing gave rise to a significant increase of plasma concentrations of MDA by 0.18 ± 0.03 μmol/L

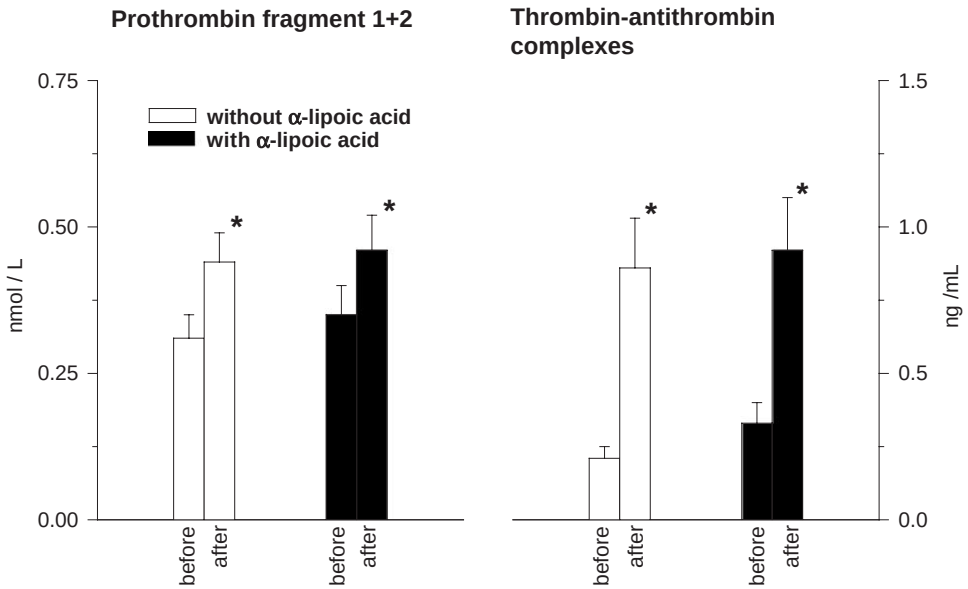

Fig. 1. The formation of thrombin in response to vigorous exercise in 10 young male individuals before and after supplementation with LA (1200 mg daily for 10 consecutive days). Plasma levels of PTF 1 + 2 and thrombin-antithrombin complexes were determined before and immediately after running on a treadmill for 1 hour to exhaustion (anaerobic threshold). Data are given as mean ± SE. Asterisk represents $P < .05$ for significant differences between preexercise and postexercise values (Wilcoxon test).

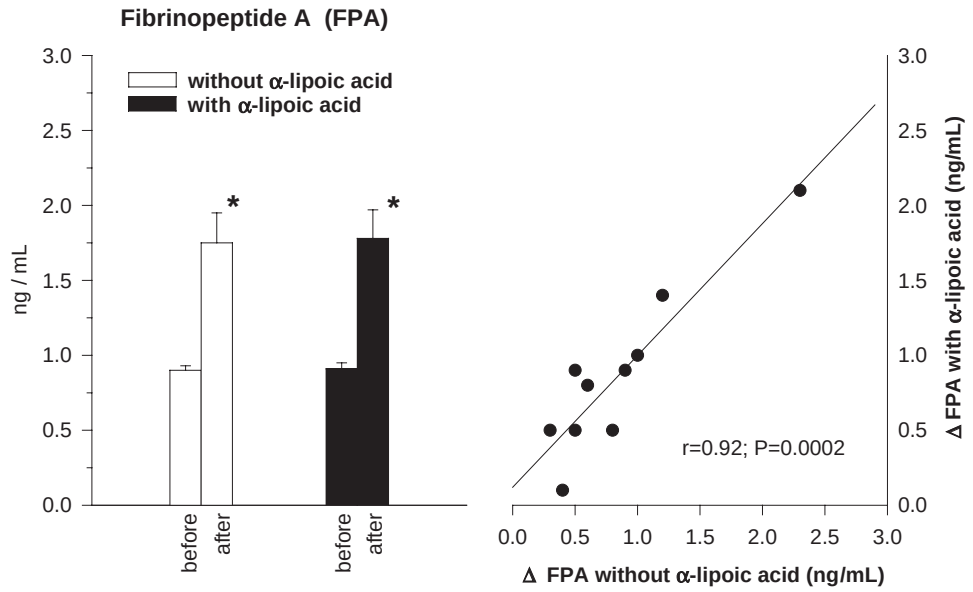

Fig. 2. Exercise-induced fibrin formation in response to vigorous exercise in 10 young male individuals before and after supplementation with LA (1200 mg daily for 10 consecutive days). Plasma levels of FPA were determined before and immediately after running on a treadmill for 1 hour to exhaustion (anaerobic threshold). Data are given as mean ± SE. Asterisk represents $P < .05$ for significant differences between preexercise and postexercise values (Wilcoxon test) (right side). Correlation analysis between exercise-induced changes of FPA (Δ-FPA) with and without supplementation with LA (Pearson correlation coefficient) (left side).

($P < .05$). Supplementation with LA had no effect on plasma levels of MDA either on baseline values or on exercise-induced changes (Table 2).

Table 2
Exercise-induced changes of fibrinogen, fibrinolytic variables, and MDA in 10 young individuals with and without supplementation with LA

	Without LA	With LA	*P*
aPTT (s)			
Before	38.4 ± 0.9	38.7 ± 1.0	NS
After	33.7 ± 0.6*	34.4 ± 0.7*	NS
ΔaPTT	−4.7 ± 0.7	−4.3 ± 0.5	NS
Fibrinogen (g/L)			
Before	2.14 ± 0.14	2.06 ± 0.09	NS
After	2.29 ± 0.15*	2.17 ± 0.09 [0.07]	NS
ΔFibrinogen	0.15 ± 0.04	0.11 ± 0.04	NS
tPA (ng/mL)			
Before	4.6 ± 0.5	4.6 ± 0.5	NS
After	23.9 ± 1.8*	23.2 ± 1.9*	NS
ΔtPA	19.5 ± 1.7	18.6 ± 1.7	NS
PAP (ng/mL)			
Before	279 ± 43	279 ± 61	NS
After	1103 ± 161*	934 ± 118*	NS
ΔPAP	825 ± 128	655 ± 73	NS
D-dimer (ng/mL)			
Before	8.2 ± 1.6	12.4 ± 3.7	NS
After	12.0 ± 2.4*	17.6 ± 6.1*	NS
ΔD-dimer	3.7 ± 1.1	5.2 ± 2.5	NS
MDA (μmol/L)			
Before	0.73 ± 0.06	0.74 ± 0.08	NS
After	0.91 ± 0.11*	0.94 ± 0.16*	NS
ΔMDA	0.18 ± 0.03	0.20 ± 0.05	NS

Mean ± SE. Δ indicates difference between preexercise and postexercise values; aPTT, activated partial thromboplastin time.

* $P < .05$ for significant differences before vs after exercise (Wilcoxon test).

3.4. Correlations

The lack of an association between LA and the activation of coagulation and fibrinolysis with exercise indicates that individual variability is comparable between both exercise tests and not affected by supplementation with LA: ΔTAT without LA and ΔTAT with LA, $r = 0.81$, $P < .01$, n = 10; ΔFPA without LA and ΔFPA with LA, $r = 0.92$, $P < .001$, n = 10; ΔD-dimer without LA and ΔD-dimer with LA, $r = 0.80$, $P < .01$, n = 10; ΔtPA without LA and ΔtPA with LA, $r = 0.63$, $P = .05$, n = 10; ΔPAP without LA and ΔPAP with LA, $r = 0.72$, $P < .05$, n = 10.

Except for a weak correlation between exercise-induced changes of MDA and PTF 1 + 2 (ΔPTF1 + 2 and ΔMDA, $r = 0.55$, $P < .05$, n = 20), no significant correlation between MDA and hemostatic/fibrinolytic variables could be established either for absolute values or for exercise-induced changes.

4. Discussion

The data demonstrate that supplementation with LA in young healthy male individuals (1) has no effect on hemostatic and fibrinolytic variables at rest, (2) does not influence changes of hemostatic and fibrinolytic variables in response to 1-hour maximal running, and (3) does not affect concentrations of MDA as an index of oxidative stress—either at rest or after exercise.

Supplementation therapy with LA is effective in inducing antioxidant effects on the biochemical level [13,14] but has also been shown to influence redox-

sensitive molecular mechanisms as the activation of NF-κB induced by cytokines, phorbolester, and advanced glycation end products [15-17]. Of special clinical interest is the potential of LA to modulate glucose metabolism in insulin-sensitive cells and tissues [18-20]. Supplementation with LA may also have some impact on blood coagulation as suggested by recent data obtained in rats with streptozotocin-induced diabetes showing a decrease in plasma levels of fibrinogen, factor VII, and von Willebrand factor after administration of LA [21].

Physical exercise is a physiological stimulus that leads to an activation of the coagulation system [1]. The mechanisms by which exercise mediates hemostatic activation are not fully understood. The increase of shear stress and the release of catecholamines most likely contribute to the activation of platelets with exercise [4,5]. This study evaluated the role of oxidative stress for exercise-induced activation of coagulation taking into account that, first, reactive oxygen species are generated with intensive physical exercise [6] and that, second, expression of tissue factor—initiating the tissue factor–dependent (formerly "extrinsic") pathway of coagulation—is regulated by the redox-sensitive transcription factor NF-κB [8].

Our data clearly indicate that supplementation with LA has no substantial effect on baseline levels of hemostatic and fibrinolytic variables in young healthy male individuals. Furthermore, LA does not influence the extent to which coagulation and fibrinolysis are activated by exhaustive running exercise.

Although LA has been suggested to be one of the most promising antioxidative supplements [22], we cannot rule out that another antioxidant would have been capable to exert effects on coagulation and fibrinolysis at rest and in response to exercise. This interpretation is supported by the finding that LA also has failed to diminish plasma concentrations of MDA. Taken together, we cannot give a clear answer as to whether oxidative stress plays a relevant role for the activation of coagulation with exercise.

4.1. Limitations of the study

Our study population includes young healthy male individuals in a good training status and raises the question as to whether supplementation with LA might exert different effects on coagulation and fibrinolysis when antioxidant defense mechanisms are compromised as, for example, in subjects with diabetes. This consideration might also apply for the lacking effects of LA on MDA because supplementation with LA has been reported to decrease concentrations of MDA in diabetes [23,24].

In summary, supplementation therapy with LA in healthy young male individuals is not effective in modulating hemostasis and fibrinolysis, either at rest or in response to exhaustive exercise. The questions on whether LA is not the appropriate antioxidative supplement to influence coagulation and whether oxidative stress is not relevant for the activation of coagulation with exercise warrant further investigations.

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