

## COMPARISON OF THE EFFECT OF $\alpha$ -LIPOIC ACID AND $\alpha$ -TOCOPHEROL SUPPLEMENTATION ON MEASURES OF OXIDATIVE STRESS

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**Abstract**—In vitro studies have shown that  $\alpha$ -lipoic acid (LA) is an antioxidant. There is a paucity of studies on LA supplementation in humans. Therefore, the aim of this study was to assess the effect of oral supplementation with LA alone and in combination with  $\alpha$ -tocopherol (AT) on measures of oxidative stress. A total of 31 healthy adults were supplemented for 2 months either with LA (600 mg/d,  $n = 16$ ), or with AT (400 IU/d,  $n = 15$ ) alone, and then with the combination of both for 2 additional months. At baseline, after 2 and 4 months of supplementation, urine for  $F_2$ -isoprostanes, plasma for protein carbonyl measurement and low-density lipoprotein (LDL) oxidative susceptibility was collected. Plasma oxidizability was assessed after incubation with 100 mM 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH) for 4 h at 37°C. LDL was subjected to copper- and AAPH-catalyzed oxidation at 37°C over 5 h and the lag time was computed. LA significantly increased the lag time of LDL lipid peroxide formation for both copper-catalyzed and AAPH-induced LDL oxidation ( $p < .05$ ), decreased urinary  $F_2$ -isoprostanes levels ( $p < .05$ ), and plasma carbonyl levels after AAPH oxidation ( $p < .001$ ). AT prolonged LDL lag time of lipid peroxide formation ( $p < .01$ ) and conjugated dienes ( $p < .01$ ) after copper-catalyzed LDL oxidation, decreased urinary  $F_2$ -isoprostanes ( $p < .001$ ), but had no effect on plasma carbonyls. The addition of LA to AT did not produce an additional significant improvement in the measures of oxidative stress. In conclusion, LA supplementation functions as an antioxidant, because it decreases plasma- and LDL-oxidation and urinary isoprostanes. © 1999 Elsevier Science Inc.

**Keywords**—Alpha-lipoate, Alpha-tocopherol, Plasma oxidation, LDL oxidation, Antioxidant, Free radicals

### INTRODUCTION

The role of  $\alpha$ -lipoic acid (LA) in energy metabolism is well documented. As lipoamide, it functions as a cofactor in the multienzyme complexes that catalyze the oxidative decarboxylation of  $\alpha$ -keto acids such as pyruvate. Recently, a great deal of attention has been focused on the antioxidant activities of LA and its reduced form, dihydrolipoic acid (DHLA). It forms a redox couple with DHLA and they may act synergistically [1–4]. They both have various properties including quenching of reactive oxygen and nitrogen species (hydroxyl radicals, peroxy radicals, superoxide, hypochlorous acid, and peroxynitrite) and metal-chelation ( $Cd^{2+}$ ,  $Fe^{3+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$ ). They are also capable of interacting with other antioxi-

dants such as ascorbate, glutathione, and ubiquinol and are thought to participate in ascorbate recycling and indirectly in the regeneration of  $\alpha$ -tocopherol (AT)[5]. LA is soluble in both lipid and aqueous environments, and is readily absorbed from the diet, transported to cells and reduced to DHLA [1,2].

Many studies have been conducted in vitro to prove its antioxidant potency. However, there are scanty data with respect to oral supplementation in humans [1–3]. In vivo, LA supplementation has been shown to prevent symptoms of scurvy in vitamin C-deficient guinea pigs and symptoms of AT deficiency in rats fed a diet lacking AT [6]. It has recently been shown that LA-supplemented old rats have improved indices of metabolic activity as well as lowered oxidative stress and damage evident with aging [7]. In humans, this compound has potential implications in diabetes. It has been successfully used to prevent diabetes-induced biologic alterations in humans such as glycation of proteins, glucose

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utilization, polyneuropathy and cataracts [8–11]. The Alpha Lipoic Acid in Diabetic Neuropathy (ALADIN) study, a 3-week multicenter, randomized, double blind, placebo-controlled trial, undertaken in Germany in 1995 demonstrated that intravenous treatment of 328 type 2 diabetic patients with 600 mg/d of alpha lipoic acid for 3 weeks significantly reduced symptoms of diabetic peripheral neuropathy [11]. Also, LA appears to protect against ischemia-reperfusion injury in animals and offers potential benefit in hepatic and neurodegenerative diseases and heavy metal poisoning [12]. Nevertheless, there are few studies demonstrating the antioxidant effect of LA in humans.

The oxidative modification of low-density lipoprotein (LDL) has been proposed to be a key early step in atherogenesis [13,14]. AT is a powerful lipid-soluble antioxidant in plasma and LDL [15]. Although decreased levels of AT are associated with increased incidence of cardiovascular disease, increased intakes are cardioprotective [16]. Supplementation with AT at doses greater than 400 IU/d has been shown to reduce LDL oxidizability in healthy volunteers [17,18].

The first aim of this study was to investigate the effect of LA supplementation in healthy volunteers on plasma and LDL-oxidation and urinary  $F_2$ -isoprostanes and to compare it to that of the well-recognized antioxidant, AT. The second purpose of this study was to evaluate the potential synergistic effect of LA and AT on the above parameters.

## MATERIAL AND METHODS

### *Study design*

This trial was a 16-week randomized trial. A total of 31 healthy subjects (15 men, 16 women) were included in the study if they fulfilled the following inclusion criteria: nonsmokers, not taking vitamin/antioxidant or estrogen supplements, thyroxin, or lipid-lowering drugs; normal plasma glucose, hepatic and renal function tests; no acute medical conditions at least 3 months prior to entry into the study. All subjects gave informed consent, and the study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas.

The subjects were randomly assigned to take either RRR AT 400 IU/d or a racemic mixture of LA, 600 mg/d for 8 weeks. Subjects returned at 8 weeks and were then given both AT (400 IU/d) and LA (600 mg/d) for an additional 8 weeks: the subjects that took AT were given both AT and LA (AT+LA), similarly, the group that took LA first was given AT in addition to LA (LA+AT). Subjects' compliance was monitored by pill count at the end of each supplementation period. Subjects followed

their regular diet and exercise during the study. They were instructed to take the supplements with meals to enhance absorption.

### *Sample collection and analysis*

Fasting blood (60 mL) was collected in EDTA-containing tubes at baseline, after 8 and 16 weeks of supplementation for the plasma lipid profile, plasma antioxidants, plasma oxidation and LDL isolation. All blood samples were collected on ice, and plasma was separated by low-speed centrifugation at 4°C. Plasma lipid and lipoprotein levels were assayed using Lipid Research Clinics methodology as described previously [17,18]. The concentration of AT in plasma and LDL was measured after extraction by ultraviolet reversed phase high-performance liquid chromatography (HPLC) as described previously [17,18]. The concentration of LA in plasma before and after supplementation was measured by the method of Sen et al. [19] in the group that was supplemented with LA only.

Plasma was subjected to 100-mM AAPH (2,2'-azobis amidinopropane hydrochloride) induced oxidation for 4 h at 37°C and arrested by addition of butylated hydroxytoluene (40  $\mu$ M) followed by refrigeration [20]. AAPH is a free radical initiator which thermally decomposes at a constant rate to produce peroxy radicals. Plasma oxidation was assayed by protein carbonyl formation. Total protein content of plasma was measured on the automated Paramax chemistry analyzer before oxidation. Plasma carbonyl content was determined before and after oxidation by enzyme-linked immunosorbent assay (ELISA) as previously described [21]. Briefly, derivatization of plasma (15  $\mu$ L) was performed by adding 45  $\mu$ L of dinitrophenylhydrazine solution (10 mM in 6-M guanidine hydrochloride, 0.5-M potassium phosphate buffer, pH 2.5). First, an anti-DNP antibody and, then, the anti-IgG peroxidase conjugate were added. The reaction was developed by the addition of trimethyl benzidine and absorbance was measured at 450 nm. Carbonyl concentration was calculated from a standard curve with oxidized BSA after subtraction of a reagent blank containing PBS. Results are expressed in nmol/mg protein.

As a measure of in vivo oxidative stress,  $F_2$ -isoprostanes were quantitated in urine. A first morning urine sample was collected from subjects at each phase and centrifuged at low speed; the supernatant was frozen without preservative at  $-70^\circ\text{C}$  until the end of the study. Creatinine was measured by Jaffe reaction on the Paramax.  $F_2$ -isoprostanes in urine was measured by a slight modification of the method of Davi et al. [22]. One milliliter of urine was acidified with 1-N HCl to pH 4 and loaded on a C18 column preconditioned with 5-mL

methanol and 5-mL ultra pure water. The eluate obtained by addition of ethyl acetate containing 1% methanol was dried under nitrogen and redissolved in enzyme immunoassay (EIA) buffer. A 1:8 dilution was performed before measurement of F<sub>2</sub>-isoprostanes by EIA using 8-epi-PGF<sub>2α</sub> as standard. (Cayman Laboratories, Ann Arbor, MI, USA). The results obtained from EIA were standardized to urinary creatinine. The method has been validated in preliminary studies where F<sub>2</sub>-isoprostane levels were significantly increased in smokers compared to matched controls (smokers: 725 ± 347 ng/mg creatinine vs. controls: 325 ± 308 ng/mg creatinine,  $p < .05$ ,  $n = 11$ ).

LDL ( $d = 1.019 - 1.063$  g/mL) was isolated by preparative ultracentrifugation in NaBr-NaCl solutions containing 1 mg/mL EDTA as described previously [17, 18]. The isolated LDL was extensively dialyzed against three exchanges (4, 4, and 2 l) of saline-EDTA at 4°C for 24 h, after which the LDL was filtered and protein content was measured by the method of Lowry *et al.*, as described previously [17,18]. After overnight dialysis against metal-free PBS, pH 7.4 (treated with Chelex 100 resin), both copper-catalyzed and metal free LDL oxidation with AAPH were undertaken. LDL (200 μg protein/mL) was oxidized in cell-free systems in PBS at 37°C with either 2.5-μmol/L copper, or 5-mM AAPH with 0.5-mM DTPA (diethylenetetramine pentacetic acid). The time course of oxidation was followed for 5 h. At 0.5, 1.0, 1.5, 2, 2.5, 3, 3.5, 4.0, and 5.0 h, oxidation was arrested by addition of 200-μmol/L EDTA and 40-μmol/L butylated hydroxytoluene and the samples were immediately refrigerated. Lag time of conjugated diene (CD) formation for copper catalyzed oxidation and lipid peroxide (LPO) formation for both systems of oxidation were computed as previously described [17,18]. LDL LPO content was determined using the ferrous oxide-xylene orange (FoX) reagent (Pierce, Rockford, IL, USA) and expressed as nmol/mg protein.

### Statistics

Results are expressed as mean ± SD for subject characteristics and mean ± S.E.M for all other data. Differences between baseline and 2 months supplementation, between 2 and 4 months of supplementation and between baseline and 4 months of supplementation, were determined by paired Student's *t*-test. A  $p$  level of .05 was taken as the threshold for statistical significance.

## RESULTS

The two randomized groups receiving either AT or LA were similar with respect to age, BMI, and lipid

Table 1. Characteristics of the Two Groups

<i>n</i>	LA 16	AT 15
Age (y)	37.5 ± 7.7	38.3 ± 6.9
BMI (kg/m <sup>2</sup> )	26.7 ± 5.3	25.3 ± 4.4
Total cholesterol (mg/dL)		
Baseline	185 ± 31	185 ± 32
2-months supplementation	184 ± 34	183 ± 26
4-months supplementation	181 ± 17	197 ± 30
Triglycerides (mg/dL)		
Baseline	89 ± 40	96 ± 52
2-months supplementation	105 ± 65	91 ± 46
4-months supplementation	104 ± 75	136 ± 99
LDL-cholesterol (mg/dL)		
Baseline	130 ± 31	125 ± 33
2-months supplementation	125 ± 37	123 ± 28
4-months supplementation	123 ± 22	123 ± 33
HDL-cholesterol (mg/dL)		
Baseline	43 ± 8	47 ± 15
2-months supplementation	44 ± 14	47 ± 13
4-months supplementation	45 ± 14	53 ± 24

Data are presented as mean ± SD.

profile at entry into the study (Table 1). After 8 weeks of supplementation either with AT or LA and 8 additional weeks of the combined supplementation, the plasma lipid profile did not change. Although plasma lipid standardized and LDL-AT concentration increased substantially with AT supplementation alone or in combination with LA ( $p < .01$ ), there was no significant effect of LA supplementation alone on plasma and LDL-AT levels (Table 2).

LA levels were measured in the plasma of 14 subjects at baseline and following LA supplementation. While it was not detectable at baseline, levels were detectable in 4 of the subjects following supplementation ( $0.48 ± 0.35$  μmol/L).

There was a significant increase in plasma carbonyls after AAPH-induced oxidation in both groups. Supplementation with LA alone significantly decreased plasma carbonyl levels after AAPH oxidation ( $p < .0001$ ), whereas AT alone did not have a significant effect (Fig. 1). The addition of LA to AT or AT to LA did not result

Table 2. Plasma and LDL Alpha-tocopherol Levels

	LA	$p^a$	AT	$p^a$
Plasma lipid standardized α-tocopherol (μmol/mmol)				
Baseline	0.91 ± 0.06		0.93 ± 0.05	
2 months	0.89 ± 0.08	NS	1.56 ± 0.10	< .001
4 months <sup>b</sup>	1.51 ± 0.11	< .001	1.24 ± 0.15	< .01
LDL α-tocopherol (nmol/mg protein)				
Baseline	6.31 ± 71		6.49 ± 0.63	
2 months	10.24 ± 2.64	NS	13.67 ± 1.34	< .01
4 months <sup>b</sup>	12.3 ± 3.38	< .01	11.69 ± 0.60	< .001

Data are presented as mean ± S.E.M.

<sup>a</sup> Compared to baseline. <sup>b</sup> AT + LA.

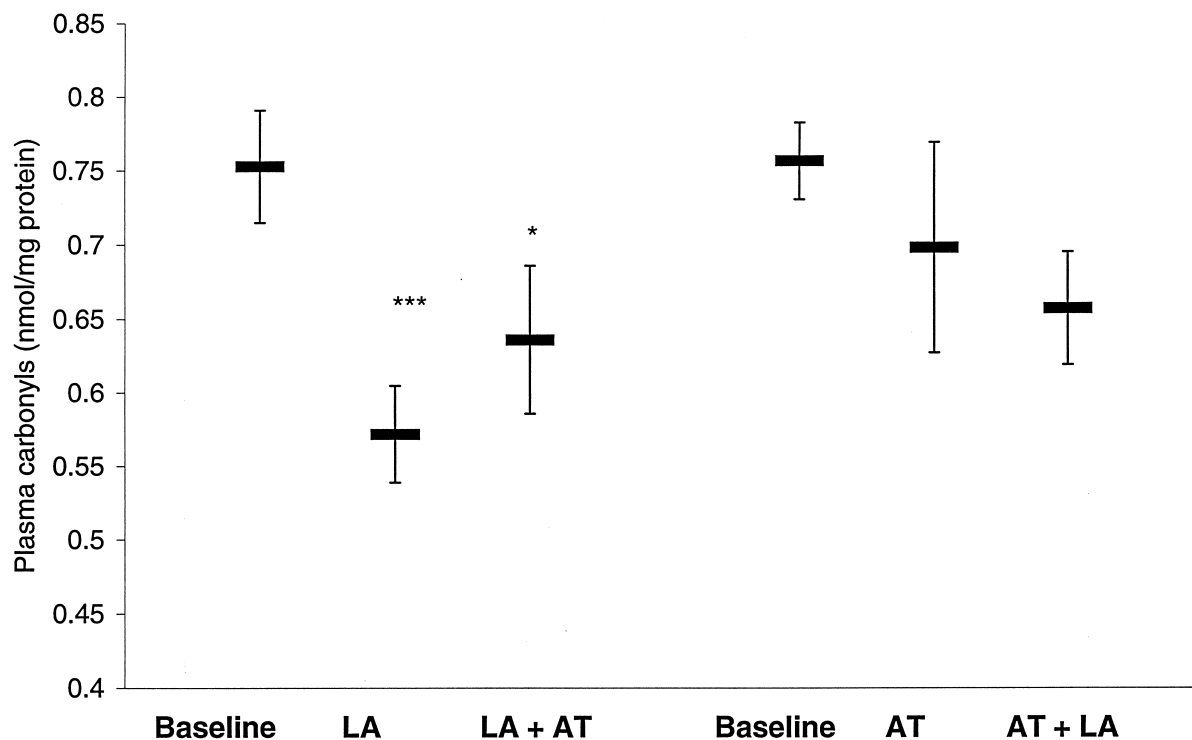


Fig. 1. Effect of supplementation on plasma oxidation as assessed by protein carbonyls. LA = after 2 months LA supplementation, AT = after 2 months AT supplementation, (LA+AT) or (AT+LA) = after 2 months of supplementation with both AT and LA after 2 months of LA or AT supplementation respectively. Data are presented as mean  $\pm$  S.E.M. \* $p < .05$ , \*\*\* $p < .001$  vs. baseline.

in an additional significant decrease in plasma carbonyl levels after AAPH oxidation.

Both LA and AT alone reduced urinary  $F_2$ -isoprostane levels, as shown in Fig. 2. Also, the addition of AT to LA resulted in a significant additional decrease in urinary  $F_2$ -isoprostanes ( $p < .05$ ). However, the addition of LA to AT did not result in any additional significant decrease in  $F_2$ -isoprostanes. With both combinations, (AT+LA) and (LA+AT), urinary  $F_2$ -isoprostane levels were significantly lower than at baseline ( $p < .01$ ) as shown in Fig. 2.

LDL oxidizability assessed by copper and AAPH-mediated oxidation was not different in the two groups at entry, as shown in Fig. 3 and Table 3. Although the lag time of LPO formation to AAPH-mediated oxidation was prolonged with supplementation with LA or AT, the increase was only significant with LA supplementation ( $p < .05$ ); the increase in lag time with AT supplementation was borderline ( $p = .06$ ). The addition of AT to LA or LA to AT supplementation did not result in an additional significant prolongation in the lag time of AAPH catalyzed LPO formation (Fig. 3). Surprisingly, as shown in Table 3, supplementation with LA alone did not significantly influence the lag time of copper catalyzed conjugated diene formation, while AT alone prolonged the lag time significantly ( $p < .01$ ). The addition

of AT to LA or LA to AT did not result in any additional significant prolongation of lag time compared to LA alone or AT alone. Both combinations, (AT+LA) and (LA+AT), resulted in a significant increase in lag time compared to baseline ( $p < .05$ ). AT supplementation resulted in a significant increase in copper-catalyzed LPO lag time ( $p < .01$ ) as shown in Table 3. Also, LA supplementation alone resulted in a significant increase in lag time ( $p < .05$ ). The combined supplementation did not significantly prolong lag time in the groups receiving LA or AT alone. However, the combined supplementation resulted in a significant prolongation of lag phase compared to baseline ( $p < .001$ ).

## DISCUSSION

LA has long been known to be an essential cofactor for energy metabolism. Recently, there has been a great deal of interest in its antioxidant properties [1–4]. LA is soluble in both aqueous and lipid environments. It has been shown in vitro to be capable of regenerating other antioxidants such as ascorbate, glutathione, ubiquinol and AT [5,23]. However, there are scanty data available on the antioxidant effect of LA supplementation in humans.

In this study, oral supplementation with LA in healthy

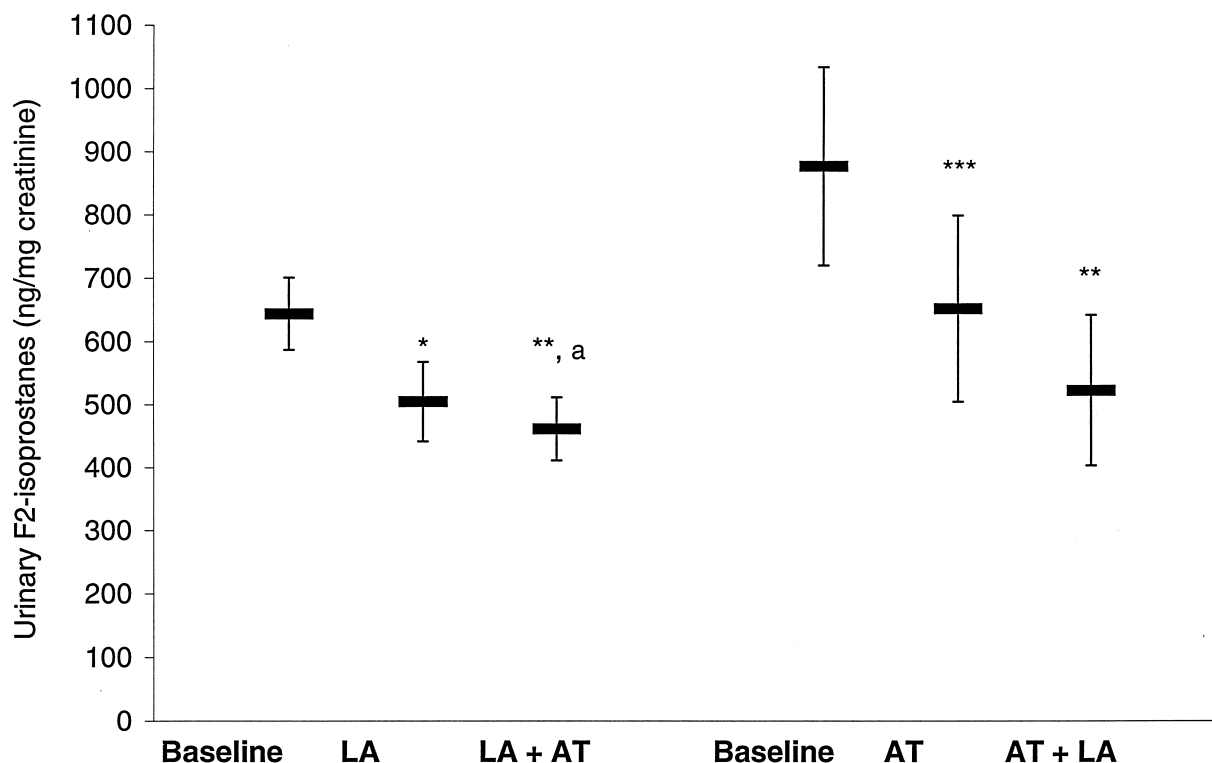


Fig. 2. Effect of supplementation on urinary F<sub>2</sub>-isoprostanes. LA = after 2 months LA supplementation, AT = after 2 months AT supplementation, (LA+AT) or (AT+LA) = after 2 months of supplementation with both AT and LA after 2 months of LA or AT supplementation, respectively. Data are presented as mean  $\pm$  S.E.M. \* $p$  < .05, \*\* $p$  < .01, \*\*\* $p$  < .001 vs. baseline; a denotes  $p$  < .05 vs. LA supplementation alone.

subjects did not affect the lipid profile and AT levels, but decreased urinary F<sub>2</sub>-isoprostanes, plasma protein carbonyls after oxidation, as well as LDL oxidizability. AT supplementation increased plasma and LDL AT levels, decreased urinary F<sub>2</sub>-isoprostanes and LDL oxidizability, but had no effect on plasma oxidation assessed by carbonyl levels. The addition of AT to LA supplementation produced an increase in AT levels in plasma and LDL and an additional decrease in urinary F<sub>2</sub>-isoprostanes. However, the addition of LA to AT supplementation did not have any additional effect on the measures of oxidative stress.

LA supplementation did not produce any change in AT levels in plasma or LDL. Although *in vitro* studies suggest that LA may act as an antioxidant through recycling vitamin C and E levels [5,23], LA supplementation had no effect on vitamin E levels in tissues of vitamin E-deficient animals [6] in accord with the present study. Also, at the entry of the study, none of the subjects were depleted in vitamin E (Table 2). Therefore, LA does not appear to act through recycling AT levels.

Although the compliance of the subjects was not questionable (as estimated by pill count and by the compliance observed by measurement of AT levels in the group taking AT), LA levels were not detectable in

the plasma samples before supplementation and only detectable in 29% of the volunteers after LA supplementation. The pharmacokinetic properties of LA were evaluated in a recent preliminary study that demonstrated an extremely short plasma half-life of about 30 min. after both oral and intravenous administration, with a nonsaturable clearance for oral doses up to 600 mg [24]. In order to reach steady state plasma concentrations, supplementation within a period of five half-lives from the previous administration may be required. This probably explains the inability to detect LA in our plasma samples, because the last LA supplement was taken at least 12 h before blood sampling. Future studies will be directed at obtaining samples at earlier time points after supplementation, to determine maximal incorporation of LA in plasma and LDL.

Plasma oxidizability by AAPH, as assessed by protein carbonyls, was significantly decreased after LA supplementation but not with AT supplementation alone. Addition of AT to LA did not additionally decrease plasma oxidizability compared with LA alone. Although *in vitro* experiments previously showed that dihydrolipoic acid had protective effects on aldehyde-induced protein modification in human plasma [25] and decreased protein carbonyl formation in BSA *in vitro* [26], there is no study



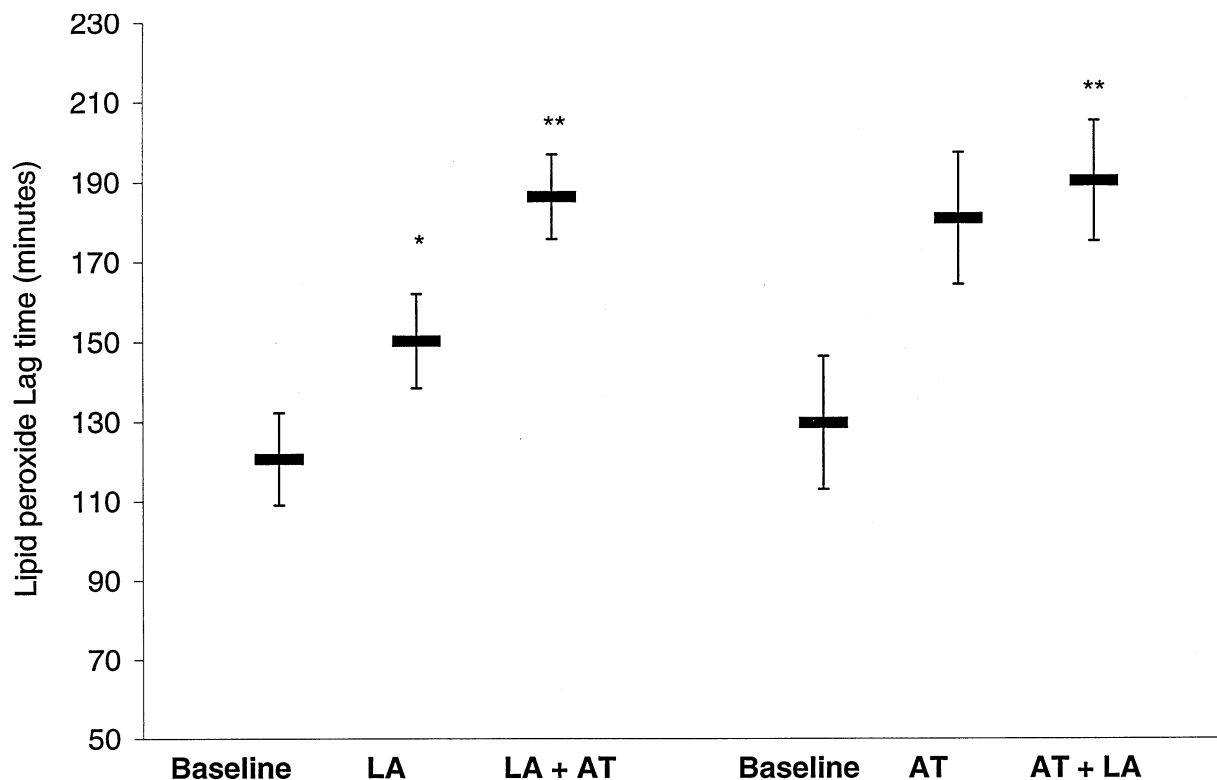


Fig. 3. Effect of supplementation on AAPH-catalyzed LDL oxidation as assessed by lag time to LPO formation. LA = after 2 months LA supplementation, AT = after 2 months AT supplementation, (LA+AT) or (AT+LA) = after 2 months of supplementation with both AT and LA after 2 months of LA or AT supplementation respectively. Data are presented as mean  $\pm$  S.E.M. \* $p$  < .05, \*\* $p$  < .01 vs. baseline.

examining the effect of LA or AT on protein oxidation in plasma following supplementation in humans. The ability of LA to decrease plasma oxidizability additionally lends support to its antioxidant properties in aqueous environments.

Previous animal studies suggest that dietary LA in

Table 3. Effect of LA and AT Supplementation on Copper-Catalyzed LDL Oxidation

	LA	$p^a$	AT	$p^a$
Conjugated dienes				
Baseline	72.9 $\pm$ 3.6		67.3 $\pm$ 3.81	
2 months	80.9 $\pm$ 6.2	NS	100.1 $\pm$ 7.8	< .01
4 months <sup>b</sup>	99.2 $\pm$ 9.6	< .05	106.5 $\pm$ 5.7	< .001
Lipid peroxides				
Baseline	88.0 $\pm$ 6.6		79.2 $\pm$ 7.3	
2 months	116.6 $\pm$ 14.7	< .05	130.0 $\pm$ 12.7	< .01
4 months <sup>b</sup>	126.8 $\pm$ 9.5	< .001	139.9 $\pm$ 10.4	< .001

Lag phase to conjugated diene and lipid peroxide formation expressed in minutes.

Data are presented as mean  $\pm$  S.E.M.

<sup>a</sup> Compared to baseline.

<sup>b</sup> (AT+LA)

animals has an antioxidant effect, both at the tissue level, as assessed by decreased lipid peroxidation and at the level of the entire animal, by suppressing symptoms of vitamin E and C deficiency [1,4,6,27]. However, no studies exist to date examining the effect of LA supplementation on markers of in vivo oxidative stress such as F<sub>2</sub>-isoprostanes, which are prostaglandinlike compounds formed from free radical-catalyzed oxidation of arachidonic acid and have been demonstrated to be valid markers of oxidative stress [22,28–31]. F<sub>2</sub>-isoprostanes are increased in patients with diabetes, hypercholesterolemia, and smokers [22,29–31]. In this study of healthy humans, LA as well as AT supplementation alone was associated with a significant decrease in F<sub>2</sub>-isoprostane levels in urine. Also, addition of AT to LA resulted in an additional significant decrease in urinary isoprostane levels compared to LA alone. This is the first study to demonstrate the antioxidant effect of LA supplementation using an in vivo marker of oxidative stress. Only a few studies have investigated the effect of antioxidants on urinary F<sub>2</sub>-isoprostanes. Reilly et al. [30] have reported no significant changes in urinary F<sub>2</sub>-isoprostane excretion after a 5-d course of vitamin E supplementa-

tion in a limited number of moderate or heavy smokers. In contrast, vitamin C (2 g/d) alone or in combination with vitamin E significantly depressed urinary F<sub>2</sub>-isoprostane in heavy smokers [30]. Two-week supplementation with vitamin E (100 and 600 mg/d) was found to reduce urinary F<sub>2</sub>-isoprostane excretion in hypercholesterolemic subjects [31]. Similar changes have been reported after high-dose vitamin E supplementation in non-insulin-dependent diabetes mellitus patients [29]. Taken together, these findings demonstrate that LA functions as an antioxidant *in vivo*, and the combination of AT and LA further enhances this effect if LA is given first. Future studies will be directed at elucidating this synergism since LA did not result in an increase in AT levels and other antioxidants were not measured.

Several lines of evidence support a proatherogenic role for oxidized LDL and supplementation with antioxidants, such as AT, have been shown to significantly decrease LDL oxidative susceptibility as evidenced by an increase in the lag phase of oxidation [13–18]. However, there is a paucity of data examining the effect of LA supplementation on LDL oxidative susceptibility in healthy humans. *In vitro*, LA/DHLA has been shown to inhibit copper-catalyzed LDL oxidation by chelating Cu<sup>2+</sup> [32]. These authors suggested that at low concentration, DHLA could reduce copper and prevent its reduction by LDL and, therefore, enhance LDL oxidation; at high concentrations, DHLA could chelate copper and thus inhibit LDL peroxidation. It was demonstrated that LA had hydroxyl radical scavenging properties, preventing apo B carbonyl formation [32], but was unable to prevent the formation of lipid peroxidation products in LDL after oxidation had been initiated with copper. Thus, we examined the effect of LA supplementation on LDL oxidizability by both metal dependent (copper) as well as metal-independent (AAPH+DTPA) systems. In this study, LA supplementation significantly increased lag time of LPO formation, after copper and AAPH catalyzed oxidation, although there was no change in lag phase of conjugated diene formation. It is unclear why LA supplementation decreased the lag time of LPO formation following AAPH- and copper-catalyzed LDL oxidation, but had no significant effect on the lag time of conjugated diene formation following copper-catalyzed LDL oxidation. However, conjugated dienes and LPOs reflect different aspects of LDL oxidation. These findings do not support the results of several *in vitro* studies suggesting that LA is a copper chelator, and inefficient in peroxyl scavenging in both aqueous and lipid environments. However, because LA is water soluble, it is unlikely that it remained on the LDL particle after LDL isolation [33]. Although the precise mechanism via which LA decreases LDL oxidation was not elucidated in the present report, LA could have enriched plasma and

LDL antioxidants other than AT or resulted in a change in LDL fatty acids and/or size resulting in decreased oxidation. Additional studies will be directed at defining the favorable effect of LA on LDL oxidation.

AT supplementation (400 IU/d) significantly prolonged LDL lag time mediated by copper. This is in accord with numerous previous studies showing that AT supplementation, at doses of  $\geq 400$  IU/d, significantly prolongs lag phase of oxidation [17,18,34,35]. The combination of LA and AT did not significantly prolong LDL resistance to oxidation additionally compared with AT alone or LA alone. Also, plasma and LDL AT did not increase with LA supplementation. These data are consistent with the findings of Kagan's *et al.* [5], which showed that LA was capable of recycling vitamin E in LDL only in the presence of vitamin C.

Therefore, in this study, LA supplementation alone significantly decreased plasma protein carbonyls, urinary isoprostanes and LDL oxidation as assessed by lag time to LPO formation, whereas AT supplementation alone had no effect on protein oxidation, but significantly decreased urinary F<sub>2</sub>-isoprostanes and LDL oxidizability. The addition of AT to LA resulted in a significant decrease in urinary F<sub>2</sub>-isoprostanes compared to LA alone, with no significant change in the other parameters of oxidative stress. Addition of LA to AT did not result in any significant change in measures of oxidative stress compared to AT alone. In conclusion, the present study provides conclusive evidence for the antioxidant effects of LA supplementation in humans on plasma- and LDL-oxidation and urinary F<sub>2</sub>-isoprostanes. Therefore, in addition to LA having the potential to prevent premature atherosclerosis via its antioxidant effect, it has the advantage in diabetic patients of improving polyneuropathy and macrovascular disease.

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#### ABBREVIATIONS

AAPH—2,2'-azobis (2-amidinopropane) hydrochloride  
 AT— $\alpha$ -tocopherol  
 BMI—body mass index  
 BSA—bovine serum albumin  
 DHLA—dihydroliipoic acid  
 EDTA—ethylenediaminetetracetic acid  
 LA— $\alpha$ -lipoic acid  
 PBS—phosphate-buffered saline