Oxidative Susceptibility of Unfractionated Serum or Plasma: Response to Antioxidants in Vitro and to Antioxidant Supplementation

Mark A. Atkin, Amy Gasper, Raj Ullegaddi, and Hilary J. Powers*

Background: The susceptibility of plasma lipids to oxidation is thought to be a factor contributing to atherogenic risk. Various groups have studied the in vitro oxidizability of isolated LDL and examined the effects of conventional antioxidants. The drawbacks associated with the isolation of LDL for evaluation of in vitro oxidizability, however, have limited the application of this measurement in large-scale studies.

Methods: We developed and evaluated an assay that can be used to directly assess the oxidative susceptibility of unfractionated serum or plasma lipids, obviating the need for isolation of lipoprotein fractions. Oxidative conditions were initiated in vitro with cuprous chloride and 2,2'-azobis(2-amidinopropane) hydrochloride. The effects of antioxidants added in vitro, and as an oral supplement, were monitored by conjugated diene formation.

Results: The addition of ascorbic acid (0–50 µmol/L) in vitro elicited a dose-dependent protective effect, increasing the lag time to oxidation ($P < 0.001$). In contrast, γ-tocopherol demonstrated prooxidant behavior at increasing concentrations (0–50 µmol/L), although we observed a decrease in the maximum rate of oxidation. Our findings are supported by the results from plasma samples of participants in a randomized antioxidant (vitamins C and E) intervention study after acute ischemic stroke. The group receiving vitamins C and E for 14 days showed an increased lag time to plasma lipid oxidation in vitro compared with the nonsupplemented group ($P < 0.05$).

Conclusion: The susceptibility of unfractionated plasma or serum lipids to oxidation in vitro offers an alternative to LDL for evaluating the efficacy of antioxidant regimens.

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Evidence suggests that oxidative modification of LDL in vivo contributes to atherogenesis, although the details of this process and the importance relative to other mechanisms are not well understood (1, 2). LDL that has undergone oxidative modification (oxLDL) has been shown to exhibit potentially atherogenic properties, including effects on platelet aggregation, foam cell formation, and proinflammatory genes (2). The susceptibility of LDL to oxidation ex vivo has been used by many groups to examine possible mechanisms whereby oxLDL might contribute to atherogenesis in vivo (3–5). The relevance of LDL oxidizability ex vivo to atherogenesis in vivo, however, is not clear. Some studies have shown a relationship between LDL oxidizability ex vivo and disease risk or vascular function (6, 7), but others have not (8), and there is a sizeable body of literature that is not compatible with an important role for oxLDL in atherogenesis. For example, issues that have yet to be resolved include the likelihood that significant LDL oxidation occurs in vivo in the presence of antioxidants, as well as the possibility that oxLDL in the vessel wall is a consequence rather than a cause of atherosclerosis (9). The oxidizability of LDL ex vivo has been shown by some groups to be inhibited by added antioxidants, notably ascorbic acid (10), γ-tocopherol, and recently, 5-methyltetrahydrofolate (11). A few studies have also demonstrated effects of antioxidant supplementation on the oxidizability of LDL ex vivo (12–14). Although these studies have produced data generally supportive of a role for conventional antioxidants in...
protecting against cardiovascular disease, the measurement of LDL oxidizability ex vivo has not emerged as a useful intermediate disease endpoint in randomized controlled trials, partly because of the lengthy procedures for LDL preparation and the need to make measurements within a few days of LDL isolation. Other issues relating to the LDL isolation process, however, raise some questions as to the in vivo relevance of observations made in vitro and the comparability of results obtained by different LDL isolation procedures. We investigated the potential value of serum or plasma lipid oxidizability in vitro as a surrogate for LDL oxidation ex vivo. We hypothesized that serum or plasma lipid oxidizability would be sensitive to conventional antioxidants added in vitro in a dose-dependent manner but also to antioxidant supplementation in vivo. To test these hypotheses, we carried out 2 studies: We first examined the effects of various concentrations of conventional antioxidants on serum lipid oxidation in vitro and then explored the effects of antioxidant intervention in stroke patients on plasma lipid oxidation in vitro. In both studies, lipid oxidation was initiated by 2 contrasting agents (metal-ion– and carbon-based).

Materials and Methods

Ethics approval for this study was obtained from the North Sheffield Local Research Ethics Committee.

In Vitro Study Design

For this study, we recruited 20 healthy individuals (7 men and 13 women) from university staff and students. A 5-mL blood sample was obtained after an overnight fast. Serum was separated and stored at −70 °C until use (within 6 weeks).

Intervention Trial Design

Stored plasma samples were available from the antioxidant arm of an intervention carried out among patients after acute ischemic stroke. Over an 18-month period, all patients admitted to a university teaching hospital with a diagnosis of acute ischemic stroke (according to the WHO criteria) were identified, and suitable patients were recruited into a randomized controlled study. After giving informed written consent, acute ischemic stroke patients admitted within 12 h of symptom onset (excluding those with cerebral or subarachnoid hemorrhage on computed tomography head scan) were randomly assigned to receive daily oral antioxidant vitamin supplements [500 mg of vitamin C plus 800 IU of vitamin E (727 mg of α-tocopherol acetate)] or no supplements for 14 days. Samples were collected into lithium heparin and stored at −70 °C for up to 6 months before analysis of lipid oxidation. Stroke patients with active gastrointestinal disease, severe medical or psychiatric illness, serum creatinine >150 μmol/L, history of gout or renal failure, current use of vitamin supplements, bleeding disorders, or inability or refusal to give consent were excluded.

Treatment group participants and controls were matched for stroke subtype and age. Plasma samples from 21 antioxidant-treated patients and 21 control patients, collected within 12 h of stroke and after 14 days of supplementation, were used in the present study.

Measurement of Serum or Plasma Lipid Oxidation

Serum (in vitro study) or plasma (intervention trial) was exposed either to 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH) at a concentration of 4 mmol/L or to copper(II) chloride at a concentration of 100 μmol/L in vitro at 37 °C and pH 7.4. Conjugated diene formation was monitored over an 8-h period. The susceptibility to oxidation was measured as the lag time to the propagation phase of lipid oxidation, defined as the intercept of the tangents of initiation and propagation. In addition, the maximum rate of the fast propagation phase was determined.

Standard Incubation Method

All in vitro oxidation reactions were performed in phosphate-buffered saline (PBS; 0.0027 M KCl, 0.137 M NaCl, pH 7.4), according to the following general procedures, based on the serum method of Schnitzer et al. (15):

Method A. PBS (900 μL; final concentration, 10 mmol/L) and antioxidant (final concentration, 0–50 μmol/L) were placed in a microcentrifuge tube. Serum or plasma (previously centrifuged at 13 000g for 1 min) was then added, followed by AAPH solution (final concentration, 4 mmol/L), and the absorbance was then measured at 37 °C at 245 nm over 480 min in 10-min cycles.

Method B. PBS (final concentration, 10 mmol/L) and antioxidant (final concentration, 0–50 μmol/L) were placed in a microcentrifuge tube. Serum or plasma (previously centrifuged at 13 000g for 1 min) was then added, followed by copper(II) chloride solution (final concentration, 100 μmol/L), and sodium citrate (final concentration, 720 μmol/L). The absorbance was then measured at 37 °C at 245 nm over 480 min in 10-min cycles. For the study of response to antioxidants added in vitro, various concentrations of ascorbate (10–50 μmol/L) and α-tocopherol (10–50 μmol/L) were added to the standard incubation mixture.

Measurement of Conjugated Dienes

A spectrophotometer (Camspec Limited) concurrently analyzed 6 samples in stopped cuvettes to measure conjugated diene formation. Measurements were carried out at 37 °C by continuous recording, with absorbance readings taken automatically every 10 min for 8 h and comparison against a reagent blank (without serum or plasma). This reaction time is sufficient to observe clear initiation and propagation phases. We monitored the oxidation reactions by measuring the absorbance of diene species formed during lipid peroxidation at 245 nm (16).
(to avoid the intense absorbance of albumin, which masks the usual diene absorbance at 234 nm). The intrabatch CV for lag time was 5.4% (n = 6).

MEASUREMENT OF ANTIOXIDANT CONCENTRATION
The concentration of ascorbic acid in plasma or serum in aliquots stored at −70 °C with 50 g/L metaphosphoric acid as stabilizer was measured by a fluorescence assay automated for the Cobas Bioautoanalyzer (Roche), according to Vuilleumier and Keck (17). Vitamin E was measured as α-tocopherol by HPLC with ultraviolet detection, as described by Thurnham et al. (18).

STATISTICAL ANALYSIS
Data were tested for normality and expressed as means (SD) or medians (interquartile ranges) as appropriate. Comparisons of gaussian-distributed data were made by ANOVA; for nonparametric data, the Kruskal–Wallis test was used. All analyses were performed with the Statistical Package for the Social Sciences (Ver. 10.0).

Results

IN VITRO STUDY
For the CuCl₂ protocol, 10 healthy participants (4 males; mean age, 32 years; range, 23–51 years) were recruited; 10 additional participants (3 males; mean age, 30 years; range, 23–51 years) were recruited to the AAPH protocol. Median (interquartile range) serum concentrations of ascorbic acid and α-tocopherol in the participants recruited to this in vitro study are shown in Table 1. Although values were higher for the CuCl₂ group than the AAPH groups, all fell within the reference intervals (19). All participants had fasting plasma cholesterol concentrations within the reference interval [mean (SD), 4.55 (0.883) mmol/L].

INTERVENTION STUDY
Plasma samples from 42 stroke patients (19 men) were used in this study. The mean age of the patients was 75 years (range, 59–91 years). Plasma antioxidant concentrations in blood samples collected within 12 h of stroke and after 14 days of antioxidant supplementation (S) or no supplementation (C) are shown in Table 2. No patients presented with a value below the accepted threshold for normality (lower limit of the reference interval) (20). Both plasma ascorbic acid and α-tocopherol concentrations showed significant increases after 14 days of supplementation (P < 0.01).

Table 1. Plasma antioxidant concentrations for healthy individuals recruited to the in vitro study.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Ascorbic acid, μmol/L</th>
<th>α-Tocopherol, μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuCl₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>94.9</td>
<td>22.3</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>80.1–97.8</td>
<td>19.4–33.8</td>
</tr>
<tr>
<td>AAPH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>58.2</td>
<td>35.5</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>48.2–63.3</td>
<td>34.0–42.1</td>
</tr>
</tbody>
</table>

Table 2. Plasma antioxidant concentrations in blood samples from elderly patients recruited to the intervention trial.

<table>
<thead>
<tr>
<th></th>
<th>Supplemented Day 0</th>
<th>Supplemented Day 14</th>
<th>Control Day 0</th>
<th>Control Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid, μmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>28.0</td>
<td>74.4b</td>
<td>33.6</td>
<td>33.6</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>16.8–53.7</td>
<td>49.3–129.1</td>
<td>21.3–63.8</td>
<td>19.0–50.4</td>
</tr>
<tr>
<td>α-Tocopherol, μmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>22.6</td>
<td>43.7b</td>
<td>21.4</td>
<td>17.7</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>20.4–28.5</td>
<td>27.9–58.8</td>
<td>17.4–27.4</td>
<td>13.2–23.7</td>
</tr>
</tbody>
</table>

* Samples were collected within 12 h of stroke (day 0) and after 14 days of either vitamin C and E supplementation (supplemented group) or no supplement (control).

b Significantly different from value at day 0 (Kruskal–Wallis), P < 0.01.

EFFECTS OF ANTIOXIDANTS ADDED IN VITRO ON SERUM LIPID PEROXIDATION
Storage of serum or plasma at −70 °C did not significantly affect lag time or maximum rate of oxidation (21). Copper-mediated serum peroxidation showed the same profile as has been well characterized for copper-mediated LDL oxidation in vitro (data not shown). These results support data presented by Schnitzer and coworkers (15, 16), showing a clear lag phase before the propagation phase of lipid peroxidation. Similarly, the profile of AAPH-induced oxidation in unfractionated serum was comparable to that of either isolated LDL as observed by Halvorsen et al. (22) or whole serum as reported by Kontush et al. (23). The response of serum oxidation to vitamins added in vitro is shown in Figs. 1 and 2. Increasing concentrations of ascorbic acid elicited a significant dose-dependent increase in lag time for both sets of initiating conditions (P < 0.001); in contrast, addition of α-tocopherol elicited a marked dose-dependent decrease in lag time when copper was used as the oxidant (P <0.001) and a modest, nonsignificant decrease when AAPH was the initiating oxidant (Fig. 1). Shown in Fig. 2A is the dose-dependent relationship between the rate of oxidation measured over the period of propagation of lipid peroxidation and the ascorbic acid or α-tocopherol concentration added, according to the oxidizing condition. In the AAPH system with ascorbic acid, the oxidation rate was constant over the concentration range (Fig. 2A); however, in the copper system, increasing the ascorbic acid concentration from 10 μmol/L led to a decrease in the oxidation rate. In contrast, addition of α-tocopherol slowed the oxidation rate in the propagation phase in a dose-dependent manner for both sets of initiating conditions (Fig. 2B).
effects of oral supplementation on plasma lipid oxidation in vitro

Median lag times for both supplemented and nonsupplemented groups at baseline and after intervention for both oxidizing conditions are shown in Table 3. The medians and interquartile ranges for the percentage change after intervention are also shown. When copper was the oxidant, the median lag time for the supplemented group increased significantly from day 0 to day 14 in contrast with the unsupplemented group, which showed no significant change over the time-course of the study. The response was significantly different between the 2 groups ($P < 0.05$). With AAPH as the initiating system, the supplemented group showed an increase in median lag time over the course of the intervention, in contrast to the unsupplemented group, which showed a decrease in lag time. The change was significantly different between the 2 groups ($P < 0.05$). There was no significant effect of supplementation on propagation rates (data not shown).

We compared median lag times for samples collected from elderly stroke patients before supplementation with lag times for the younger, healthy participants. Lag times were significantly longer for samples collected from the younger, healthy participants for both copper ($P < 0.001$) and AAPH ($P < 0.001$) initiating conditions. To examine the possibility that this difference was related to a lower concentration of ascorbic acid in the plasma of elderly people compared with that in healthy younger people, we performed a regression analysis for lag time vs ascorbic acid concentration for young and old participants combined, with baseline values in the elderly group and values in the absence of added antioxidant for the younger, healthy group. Analysis revealed a statistically significant correlation ($r = 0.258; P = 0.023$).

**Discussion**

EFFECTS OF VITAMIN C ADDED IN VITRO

Increasing the ascorbate concentration produced a dose-dependent increase in lag time in both oxidizing sys-

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Fig. 1. Effect of added ascorbic acid (A) or α-tocopherol (B) on lag time to oxidation of serum lipids in copper (●) and AAPH-based (▲) oxidizing systems.

Values are the mean (SD; error bars). Effect of ascorbic acid concentration on lag times (ANOVA): $P < 0.001$ for both copper and AAPH systems. Effect of α-tocopherol concentration on lag time (ANOVA): $P < 0.001$ for copper; not significant for AAPH.

Fig. 2. Effect of added ascorbic acid (A) or α-tocopherol (B) on maximum rate of oxidation for copper (●) and AAPH (▲) oxidizing systems.

Values are the mean (SD; error bars) in absorbance units (AU)/min. Effect of ascorbic acid concentration on mean rate of oxidation (ANOVA): $P < 0.001$ for copper; not significant for AAPH. Effect of α-tocopherol concentration on mean rate of oxidation (ANOVA): $P < 0.01$ for both copper and AAPH.
tems. This protective effect has been observed in isolated LDL preparations exposed to copper (12, 24) and by Schnitzer’s group for unfractionated serum (15, 16). Ascorbic acid shows this protective effect toward serum lipids regardless of the initiating oxidant because in our study we used both a metal ion and an “organic” source to generate free radicals. The free-radical–lowering activity of ascorbic acid has been well documented (12, 15). In the copper-based system, the in vitro addition of ascorbate also decreased the rate of oxidation during the propagation phase, whereas this decrease was not evident in the AAPH system. Previous studies have shown that the protective effect of ascorbic acid can persist even after it has been completely oxidized, although the mechanisms involved are not completely understood (25).

**EFFECTS OF VITAMIN E ADDED IN VITRO**

Addition of α-tocopherol to either the copper-mediated or AAPH system shortened the lag time to the propagation phase of oxidation, indicating a prooxidant activity. The duality of oxidant behavior displayed by α-tocopherol in different lipid systems has been reported by others (26, 27), and the mechanistic basis of this behavior has been explained in some detail (28, 29). When LDL is exposed to a strong oxidizing agent, α-tocopherol is depleted rapidly and the formation of lipid peroxides is slow. In this scenario, α-tocopherol is acting as an antioxidant (28). Several groups have reported antioxidant activity of α-tocopherol toward lipids in isolated LDL (30, 31). Under weaker oxidizing conditions, α-tocopherol is oxidized less rapidly, and tocopherol-mediated peroxidation, in which the α-tocopherol is successively oxidized, mainly to the tocopheroxyl radical (α-TO’), and reduced, would be expected to occur. The α-TO’ radicals generated react with lipid, however, and lipid peroxide formation is thus more rapid (28). Various factors influence the behavior of α-tocopherol, including the concentration of preformed lipid peroxides in the lipid substrate, which exert an effect dependent on the relative concentration of α-tocopherol, the lipid hydroperoxide, and the nature and concentration of the initiating radical species (21). Lipid hydroperoxides may reduce the α-TO’ radical but in the process generate peroxyl radicals, which are involved in the propagation of lipid oxidation. The availability of ascorbate may also influence the effects of added α-tocopherol on lipid oxidation because ascorbate regenerates the reduced tocopherol, thereby removing potentially damaging α-TO’ radicals (24, 32). In our study, the prooxidant effect of α-tocopherol was more pronounced in the copper-mediated system than in the AAPH system. This difference probably reflects the milder oxidizing environment provided by copper chloride in the presence of sodium citrate vs that provided by 4 mmol/L AAPH (33, 34). In both oxidant systems, increasing the concentration of α-tocopherol caused a decrease in the mean rate of oxidation in the propagation phase. This result is consistent with the results reported by Kontush et al. (23), who showed that 30 μmol/L α-tocopherol decreased the oxidation rate of plasma lipids in the presence of copper.

**RELEVANCE OF ASSAY TO CONDITIONS IN VIVO**

The susceptibility of serum lipids to 2 different oxidizing conditions in vitro is relatively rapid, reproducible, and sensitive to added conventional antioxidants. The mechanisms of oxidation of lipids in lipoproteins in vivo are not fully understood, and the species that initiate oxidation are not known with any certainty. In addition, there is still uncertainty about the sites of lipid oxidation in vivo. Although the presence of free transition metal ions at the site of lipid oxidation is not clear, it has been suggested that damage to the arterial wall may increase the availability of such species (35), thus promoting lipid oxidation at this site. In contrast, the carbon-centered radical that AAPH generates may more closely represent

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**Table 3. Median (interquartile range) lag times for samples collected from elderly people on day 0 (within 12 h of stroke) and after 14 days of supplement or placebo, for 2 oxidizing conditions.**

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>Lag time, min</th>
<th>ΔLag time, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong></td>
<td><strong>Day 14</strong></td>
<td><strong>%</strong></td>
</tr>
<tr>
<td>AAPH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplemented group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>51.2</td>
<td>57.9</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>47.1–60.9</td>
<td>49.6–62.8</td>
</tr>
<tr>
<td>Unsupplemented group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>53.2</td>
<td>47.5</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>44.6–64.2</td>
<td>35.0–57.2</td>
</tr>
<tr>
<td>CuCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplemented group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>36.7</td>
<td>48.7</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>32.0–40.8</td>
<td>37.6–60.8</td>
</tr>
<tr>
<td>Unsupplemented group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>45.4</td>
<td>49.3</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>41.0–46.4</td>
<td>40.3–53.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Lag times, with CuCl<sub>2</sub> or AAPH used as oxidant, at day 0 (within 12 h of stroke) and after 14 days.

<sup>b</sup>Change over the course of intervention significantly different from unsupplemented group (Kruskal Wallis), P <0.05.
the true physiologic case and provides a useful alternative to simple inorganic metal salts. The concentrations of α-tocopherol and ascorbic acid used are readily achievable in vivo, but the antioxidant/lipid ratio in the system is high; thus, although the results demonstrate “proof of principle”, further studies with lower antioxidant concentrations are needed. Effects observed in the intervention trial were much weaker, presumably reflecting the lower antioxidant/lipid ratio achievable in plasma. Various methodologic issues limit our interpretation of ex vivo LDL oxidation studies. The methods for preparation of LDL are diverse, but many use long centrifugation procedures that may enhance lipid peroxidation. The presence of lipid peroxides in LDL preparations has a profound effect on their behavior on exposure to oxidizing species (36). Some groups have used antioxidants during the preparation process, but these must be removed before study of LDL oxidation, and the processes involved may influence the composition of the LDL particles. Lipoproteins other than LDL, such as HDL, are also susceptible to oxidation, and the study of LDL in isolation may not fully represent the situation in vivo. Serum oxidizability in vitro thus represents a useful alternative to similar measurements made on isolated LDL and should be of particular value to large-scale intervention trials as an intermediate disease endpoint.

**INTERVENTION STUDY**

We applied the methods for the evaluation of serum lipid oxidizability in vitro to an intervention trial in stroke patients. Results showed a modest but statistically significant improvement in resistance of plasma lipids to oxidative stress in vitro after supplementation with α-tocopherol and ascorbic acid, compared with an untreated group. The role of radical species in stroke has been discussed elsewhere (37). A stroke involving cerebral infarction initiates a complex cascade of events in the surrounding tissue, and free-radical–mediated oxidative damage is thought to play an important role in the pathogenesis of cerebral ischemia (37). During this process, free radicals are liberated from a variety of sources (38), and hydroxyl, peroxynitrite, and superoxide radicals are believed to initiate lipid peroxidation, causing damage to plasma and mitochondrial membranes (39).

There is evidence suggesting that poor α-tocopherol and vitamin C status might enhance oxidative damage associated with ischemia and that supplementation can provide protection against such damage. Leinonen et al. (40) reported an inverse association between the plasma concentrations of ascorbate and α-tocopherol and the extent of neurologic impairment after ischemic stroke. Cherubini et al. (41) found evidence of reduced antioxidant concentrations in ischemic stroke patients compared with age- and sex-matched controls.

For both oxidation systems in this study, lag times were significantly shorter than for healthy, younger participants and were positively correlated with ascorbic acid concentrations, which were lower in the elderly group than in the healthy, younger group ($P < 0.01$). Improvements in resistance of plasma lipids to peroxidation in vitro after a short period of supplementation, however, did not increase lag times to within the (mean ± 2 SD) range of values seen in healthy, younger participants. A longer period of intervention might have done so but would be less relevant to the short-term clinical outcome of these patients. The observed difference in lag time between the young and elderly groups could also be a function of pathology, as has been shown for lipoproteins from plasma collected from Alzheimer disease patients compared with controls (42). This group also showed an effect on oxidizability of plasma lipids of a combined α-tocopherol and ascorbic acid supplement for 1 month in patients with Alzheimer disease (43).

In conclusion, we evaluated the sensitivity of lipid peroxidation in unfraccionated human serum and plasma in vitro to conventional antioxidants added either directly to an in vitro system or as an oral supplement. Initiation of lipid oxidation mediated by either copper or a carbon-based radical-generating system was delayed by added ascorbic acid in a dose-dependent manner. In contrast, α-tocopherol elicited no such effect and accelerated the onset of lipid peroxidation mediated by copper. These results support data generated by studies of isolated LDL in vitro. The susceptibility of plasma lipids to peroxidation in vitro was decreased in stroke patients by a short period of supplementation with vitamins E and C compared with unsupplemented patients. Measurement of the susceptibility of serum or plasma lipids to peroxidation in vitro offers a rapid, reproducible alternative to the study of isolated LDL for large-scale human studies.

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**References**


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