Increased serum and low-density-lipoprotein antioxidant potential after antioxidant supplementation in endurance athletes

Tommi J Vasankari, Urho M Kujala, Tuula M Vasankari, Timo Vuorimaa, and Markku Ahotupa

ABSTRACT We studied the effect of antioxidant supplementation on acute exercise-induced lipid peroxidation and antioxidant potential measured in serum and low-density-lipoprotein (LDL) samples. Eight endurance athletes repeated a 31-km running exercise twice with an interval of 4 wk. During the 4 wk before the runs, the subjects took in a single-blind randomized order either a combination of antioxidant supplements (the antioxidant trial; 294 mg vitamin E, 1000 mg vitamin C, and 60 mg ubiquinone daily) or placebo (the placebo trial). Venous blood samples were taken before and immediately after the 31-km run in both trials. Antioxidant supplementation raised the LDL antioxidant potential (TRAP) (40% and 30%, P = 0.0031), serum TRAP (9% and 10%, P = 0.0037), and serum α-tocopherol concentration (by 59% and 66%, P = 0.0004) in both pre- and postexercise samples, respectively. The supplementation did not, however, affect the concentration of LDL diene conjugation (DC) or of serum DC. Physical exercise increased serum DC (by 18% and 10%, P = 0.0004) but not LDL-DC, and the quantity of the increment of serum DC was not affected by antioxidant intervention. The major cause for the increased LDL-TRAP and serum TRAP after antioxidant supplementation is apparently the elevation of the serum α-tocopherol concentration. Am J Clin Nutr 1997;65:1052–6.

KEY WORDS Antioxidant potential, diene conjugation, lipid peroxidation, physical exercise, ubiquinone, vitamin C, vitamin E, men

INTRODUCTION Low-density lipoprotein (LDL) that has undergone oxidative modification may be more atherogenic than native LDL (1, 2). The oxidized LDL particles (and certain other forms of LDL) are ligands for the scavenger receptors on macrophages and can therefore convert them into the cholesterol-loaded foam cells that are characteristic of the earliest atherosclerotic lesions, the fatty streaks (3). Furthermore, epidemiologic studies indicate that LDL oxidation is associated with the severity of coronary atherosclerosis (4). The oxidative modification of LDL is assumed to occur primarily in the intima of the artery, in microdomains sequestered from antioxidants in plasma (2). However, a major part of the LDL entering the artery wall will reenter circulation without undergoing degradation and there is evidence for the existence of circulating oxidized LDL (5, 6).

There is epidemiologic evidence that high doses of vitamin E may reduce the risk of coronary artery disease (7). In addition, coantioxidants can make vitamin E an even more efficient antioxidant for LDL (8). Cosupplementation with ubiquinone may prove to be particularly efficient because this compound becomes incorporated into LDL, thereby providing antioxidant protection at the required site (8). Addition of ascorbate to LDL undergoing oxidation mediated by aqueous peroxyl radicals may also halt α-tocopherol consumption and hence LDL oxidation, as indicated by the inhibition of cholesteryl ester hydroperoxide formation (8).

Antioxidant supplementation may also protect tissues against exercise-induced oxidative stress (lipid peroxidation), but the existing data on humans are contradictory (9–11). In the present study we followed the effect of 4 wk of antioxidant cosupplementation (a combination of vitamin E, vitamin C, and ubiquinone) on basal and acute exercise-induced lipid peroxidation and antioxidant potential measured in serum and in the serum LDL fraction. LDL oxidation was measured by a method that determines the concentrations of conjugated dienes extracted from LDL (LDL-DC) (12). The antioxidant potential of LDL and serum samples was estimated in vitro by their potency to resist 2,2′-azobis(2-amidinopropane) HCl (ABAP)–induced peroxidation (12).

SUBJECTS AND METHODS Subjects A group of eight healthy competitive male endurance runners was examined. The subjects gave written informed consent after having been explained the purpose, possible risks, and stress associated with the study. The mean age of the subjects was 31.0 y (range: 25–39 y), height was 173.5 cm (168–181 cm), weight was 62.3 kg (54–76 kg), training history

1 From the Paavo Nurmi Center, Sports Medical Research Unit; the MCA Research Laboratory, Department of Physiology, University of Turku, Turku, Finland; the Finnish Sports Institute, Vierumäki, Finland; the Unit for Sports and Exercise Medicine, Institute of Biomedicine, University of Helsinki, Helsinki; and the Department of Pulmonary Medicine and Allergology, Turku University Hospital, Turku, Finland.
2 Supported in part by the Finnish Olympic Committee and the Juho Vainio Foundation, Finland.
3 Address reprint requests to TJ Vasankari, Department of Physiology, University of Turku, Kiinnonmyllynkatu 10, FIN-20520 Turku, Finland. Accepted May 24, 1996.
was 13.8 y (6–21 y), and marathon record was 2 h, 22 min, and 32 s (2.12–2.37). The study was conducted according to guidelines of the ethical committee of Turku University and Turku University Hospital and the Declaration of Helsinki.

Study design

The subjects repeated twice a 31-km run (3 km warming up, 4 km at a speed of 3.20 min/km, 20 km at a speed of 4.00 min/km, and 4 km at a speed of 3.20 min/km) at an interval of 4 wk. During the 4 wk before the tests, the subjects consumed in a single-blind randomized order either a combination of antioxidant supplements (the antioxidant trial) or placebo (the placebo trial). The antioxidant supplementation contained 294 mg d-α-tocopheryl acetate (Tokovitain, d-α-tocopherol acetate; Orion, Espoo, Finland), 1000 mg vitamin C (Ascorbin, ascorbic acid; Orion), and 60 mg ubiquinone (Coenzyme Q10, ubidecarenon; RP Scherer Ltd, Wiltshire, United Kingdom) per day. The amount of supplementation of d-α-tocopherol acetate and ascorbic acid was 27 and 17 times the RDAs of vitamins E and C, respectively (13).

During the 31-km runs, the mean heart rate and blood lactate in the both trials were as follows: after 3 km, 125 beats/min and 1.2 mmol/L; after 7 km, 163 beats/min and 2.8 mmol/L; after 27 km, 146 beats/min and 2.1 mmol/L; and after 31 km, 170 beats/min and 3.6 mmol/L, respectively. There were no differences in the mean heart rate and blood lactate between the trials.

On the days preceding the test, the subjects consumed a normal Finnish diet. During the 4 wk before the runs, the mean (range) training distance in the antioxidant and placebo trial were 540 km (390–825 km) and 541 km (408–750 km), respectively. On the test days, the subjects had a light mixed breakfast without caffeine drinks at 0900. During the runs, the subjects were only allowed to drink water ad libitum. The exercise was carried out between 1300 and 1600. Antecubital venous blood samples were taken before warming up and immediately after the 31-km runs. The serum was separated by centrifugation at 2000 × g for 10 min at 4 °C and stored at −70 °C until analyzed.

Analytical methods

LDL oxidation was measured by the amount of LDL-DC, which was measured by a method that was validated recently and reported in detail (12). In brief, serum LDLs were isolated by precipitation with buffered heparin (14). The amount of peroxided lipids in serum and LDL samples (serum DC and LDL-DC) was determined by the degree of conjugated diene double bonds (diene conjugation; DC) in vivo. Lipids were extracted from the serum and LDL samples were extracted by a mixture of chloroform and methanol (2:1), dried under nitrogen, redissolved in cyclohexane, and analyzed spectrophotometrically at 234 nm.

Use of the precipitation method described did not totally rule out the possible contribution of small amounts of lipoproteins other than apolipoprotein B (12). On the other hand, at least under certain circumstances, the oxidized very-low-density lipoprotein (VLDL) too seems to be implicated in the development of atherosclerotic lesions (15).

The antioxidant potential of the serum and LDL samples was estimated in vitro by their ability to resist ABAP-induced peroxidation (12). This total peroxyl radical trapping antioxidant potential (serum TRAP and LDL-TRAP) was measured as follows: 0.45 mL 0.1 mol sodium phosphate buffer/L (pH 7.4) containing 0.9% NaCl, 0.02 mL 120 mmol linoleic acid/L, 0.05 mL luminol (0.5 g/L), and serum and LDL samples (20 μL serum) were mixed in the cuvette and the assay was initiated by adding 0.05 mL ABAP (83 g/L); the chemiluminescence in duplicate cuvettes at 37 °C was measured until a peak value for each sample was detected. The peroxyl radical trapping capacity was defined by the half-peak time point. Trolox (Aldrich, Milwaukee) served as the standard radical scavenger. To get an estimate of the relative antioxidant power of the LDL preparations, the results were expressed in relation to the cholesterol concentration of the preparations.

Serum concentrations of α-tocopherol (16), retinol (16), and ubiquinol-10 (17) were analyzed by standard HPLC procedures with ultraviolet detection, although it is known that more accurate measurements for ubiquinol require the use of an electrochemical detector. We also measured the concentration of reduced and oxidized ubiquinol-10 and calculated the ratio of reduced to oxidized ubiquinol-10. The reference ranges measured by us for human adults were as follows: LDL-DC, 24.9–60.0 μmol/L; LDL-TRAP, 18.6–33.2 μmol/mmol cholesterol; serum DC, 35.2–91.9 μmol/L; serum TRAP, 750–1340 μmol/L; serum α-tocopherol, 17.9–46.3 μmol/L; serum retinol, 1.19–2.90 μmol/L; and serum ubiquinol-10, 0.40–2.40 μmol/L.

Statistical analyses

Statistical analyses were performed with BMDP 2V Statistical Software (SPSS Inc, Chicago). An a priori P value for statistical significance of 0.05 was used. A two-way ANOVA with two repeated measures over both factors [exercise (preexercise/postexercise) × treatment (antioxidant/placebo)] was used to examine the exercise and treatment effects on the variables. There were no significant interactions for exercise × treatment. In case of significant exercise or treatment effects, the postexercise samples were compared with the preexercise samples and the antioxidant samples were compared with the placebo samples; a matched-pair t test was used in the computations. The concentrations are expressed as means ± SDs.

RESULTS

The antioxidant treatment significantly increased LDL-TRAP (P = 0.0031), serum TRAP (P = 0.0037), and serum α-tocopherol (P = 0.0004) values, and decreased the ratio of reduced to oxidized ubiquinol-10 (P = 0.0081); however, LDL-DC (P = 0.91), serum DC (P = 0.78), serum retinol (P = 0.39), and serum ubiquinol-10 (P = 0.62) concentrations were not affected. The preexercise and postexercise concentrations of LDL-TRAP increased by 40% and 30%, of serum TRAP by 9% and 10%, and of serum α-tocopherol by 59% and 66% for the active treatment and placebo, respectively (Table 1 and Table 2). Also, the preexercise and postexercise concentrations of the ratio of reduced to oxidized ubiquinol-10 decreased by 26% and 38%, respectively, in the antioxidant trial compared with the placebo trial.

There were significant exercise-induced increases in concentrations of serum DC (P = 0.0004), serum TRAP (P =
TABLE 1
Concentrations of LDL-diene conjugation (LDL-DC) and antioxidant potential (LDL-TRAP), and serum DC and TRAP before and after the 31-km run in the antioxidant and placebo groups

<table>
<thead>
<tr>
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<th>Before run</th>
<th>After run</th>
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<tr>
<td>Serum DC (μmol/L)</td>
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<tr>
<td>Antioxidant</td>
<td>70.9 ± 12.4</td>
<td>83.4 ± 15.1</td>
</tr>
<tr>
<td>Placebo</td>
<td>72.8 ± 10.7</td>
<td>79.9 ± 13.5</td>
</tr>
<tr>
<td>LDL-DC (μmol/L)</td>
<td></td>
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<tr>
<td>Antioxidant</td>
<td>55.9 ± 12.3</td>
<td>58.0 ± 14.2</td>
</tr>
<tr>
<td>Placebo</td>
<td>56.8 ± 13.1</td>
<td>56.0 ± 13.7</td>
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<tr>
<td>Serum TRAP (μmol/L)</td>
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<td></td>
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<tr>
<td>Antioxidant</td>
<td>915 ± 111</td>
<td>1097 ± 100</td>
</tr>
<tr>
<td>Placebo</td>
<td>836 ± 68</td>
<td>998 ± 50</td>
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<tr>
<td>LDL-TRAP (μmol/mmol cholesterol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant</td>
<td>31.1 ± 5.3</td>
<td>28.1 ± 5.2</td>
</tr>
<tr>
<td>Placebo</td>
<td>22.2 ± 5.5</td>
<td>21.6 ± 2.7</td>
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1 SD; n = 8. TRAP, total peroxyl radical trapping antioxidant potential.
2-4 Significantly different from before run: 2 P < 0.001, 3.6 P < 0.05. 4 P < 0.01.
5 Significantly different from antioxidant: 5 P < 0.05, 6 P < 0.01.

0.0002), serum α-tocopherol (P = 0.0007), and serum retinol (P = 0.0072), LDL-DC (P = 0.072), LDL-TRAP (P = 0.097), and serum ubiquinol-10 (P = 0.95) values and the ratio of reduced to oxidized ubiquinol-10 (P = 0.82) were not affected by exercise. Serum DC rose 18% and 10%, serum TRAP by 20% and 19%, serum α-tocopherol by 21% and 17%, and serum retinol by 7% and 6% in subjects receiving active therapy and placebo, respectively (Tables 1 and 2).

DISCUSSION
Vitamin E (α-tocopherol) is the principal lipid-soluble antioxidant in biological systems. Vitamin C (ascorbic acid) acts as an antioxidant apparently by regenerating α-tocopherol (18), and ubiquinone seems to spare α-tocopherol when both antioxidants are present on the same liposomal membranes (19). Therefore, for the purposes of the present study, we chose a combination of antioxidants—vitamin E, vitamin C, and ubiquinone—to maximize the biological antioxidant effect of exogenously administered drugs.

The antioxidant treatment used in this study increased substantially the concentration of serum α-tocopherol. The increase varied from 7.3 to 19.9 μmol/L (mean: 12.3 μmol/L) in the preexercise samples. Unexpectedly, serum ubiquinol-10 concentrations did not rise despite daily doses of 60 mg ubiquinol in combination with other antioxidants. This may have been because of impaired intestinal absorption of ubiquinone by interaction through the relatively high simultaneous dose of vitamin E (294 mg/d). On the other hand, the daily amount of ubiquinone may have been too low because it has been shown that 100 mg ubiquinol-10 increases the concentration of LDL ubiquinone about fourfold (8). The concentration of ubiquinone in LDL was not measured in the present study. The unchanged serum ubiquinol-10 concentration after antioxidant supplementation may also have been explained by the relatively high baseline concentration of serum ubiquinol-10.

The reduced form of ubiquinone is the first antioxidant consumed when LDL is exposed to oxidants (20, 21). In the present study, the ratio of reduced to oxidized ubiquinone was decreased by antioxidant supplementation, indicating increased consumption of reduced ubiquinone. This may indicate that the consumption of reduced ubiquinone in LDL rose, but, still, the reason for the antioxidant supplementation–related shift in the ratio of reduced to oxidized ubiquinol-10 remains unexplained.

Antioxidant supplementation improved the antioxidant potential of both serum and, even more so, of LDL samples. There was a simultaneous antioxidant treatment–related increase in the serum concentration of α-tocopherol. We showed previously that there was a significant correlation between serum α-tocopherol and serum TRAP in pre- and postexercise samples of keep-fit marathon runners (preexercise: r = 0.47; 95% CI: 0.064, 0.75; postexercise: r = 0.52; 95% CI: 0.13, 0.77) (22). In the present study the postexercise correlation between serum TRAP and serum α-tocopherol was even stronger, but because of the smaller number of subjects (n = 8) than in our earlier study (n = 22) the correlation was not significant (both trials: r = 0.64; 95% CI: −0.12, 0.93).

Hence, the main reason for increased LDL-TRAP and serum TRAP concentrations after antioxidant supplementation was the increase in serum α-tocopherol concentration. In accordance, the more pronounced elevation of the antioxidant potential in LDL is probably because a major part of α-tocopherol in plasma is transported in LDL (23). The current finding of increased LDL antioxidant potential supports the concept that α-tocopherol is crucial for the prevention of LDL oxidation and cardiovascular diseases (7). The only larger controlled study of the effects of α-tocopherol on coronary artery disease reported a small nonsignificant reduction in coronary artery disease mortality (relative risk = 0.95) and a significant reduction in the incidence of angina pectoris (relative risk = 0.91) with a very low dose of vitamin E (50 mg/d) (24, 25).

The amount of antioxidant supplementation used in this study did not affect the concentrations of serum and LDL-DC. Nor did the supplementation attenuate the amount of acute

TABLE 2
Concentrations of serum α-tocopherol, retinol, and ubiquinol-10 and the ratio of reduced to oxidized ubiquinol-10 before and after the 31-km run in the antioxidant and placebo groups

<table>
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<tr>
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<th>Before run</th>
<th>After run</th>
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<tbody>
<tr>
<td>Serum α-tocopherol (μmol/L)</td>
<td></td>
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<tr>
<td>Antioxidant</td>
<td>36.7 ± 7.7</td>
<td>44.3 ± 11.7</td>
</tr>
<tr>
<td>Placebo</td>
<td>24.4 ± 5.6</td>
<td>28.5 ± 6.7</td>
</tr>
<tr>
<td>Serum retinol (μmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant</td>
<td>1.94 ± 0.12</td>
<td>2.07 ± 0.23</td>
</tr>
<tr>
<td>Placebo</td>
<td>2.03 ± 0.13</td>
<td>2.15 ± 0.14</td>
</tr>
<tr>
<td>Serum ubiquinol-10 (μmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant</td>
<td>2.01 ± 0.61</td>
<td>1.95 ± 0.55</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.82 ± 0.90</td>
<td>1.87 ± 0.81</td>
</tr>
<tr>
<td>Reduced: oxidized serum ubiquinol-10</td>
<td></td>
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<tr>
<td>Antioxidant</td>
<td>1.51 ± 0.54</td>
<td>1.33 ± 0.25</td>
</tr>
<tr>
<td>Placebo</td>
<td>2.03 ± 0.45</td>
<td>2.15 ± 0.24</td>
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1 SD; n = 8. 2-4 Significantly different from before run: 2 0.001 < P < 0.01, 3 P < 0.001, 4 P < 0.05. 5-7 Significantly different from antioxidant: 5 P < 0.01, 6 P < 0.05, 7 P < 0.001.
exercise-induced lipid peroxidation in serum. As in a previous study, prolonged acute exercise raised serum DC but did not alter the LDL-DC concentration (22). The reason why DC increases in serum but not in LDL with exercise may be partly explained by increased HDL concentrations after exercise (26). LDL-DC may act as an indicator of LDL oxidation and thus be an atherogenic modification of LDL, but it does not change during acute oxidative stress, such as physical exercise. Rather, measurement of serum DC is a useful method to estimate changes in acute physiologic oxidative stress (27). On the other hand, veteran endurance athletes have been found to have low LDL oxidation compared with age-matched control subjects (28).

The serum TRAP value rose during exercise in the antioxidant trial as well as in the placebo trial (20% and 19%, respectively). At the same time, the serum α-tocopherol concentration rose (by 21% and 16%, respectively) and a minor exercise-induced increase was recorded for serum retinol too (7% and 6%, respectively). Serum α-tocopherol is known to rise in conjunction with intensive exercise, and it may be due to the increased mobilization of α-tocopherol into blood (29, 30). The exercise-induced increase in serum α-tocopherol may also have been due to the increased flux of fatty acids through the liver, which stimulates secretion of VLDL, which are enriched in α-tocopherol by the tocopherol transfer protein (23). On the other hand, the increased concentration of α-tocopherol may also be caused by a shift of water from plasma to muscles during exercise, which can result in a higher concentration of blood constituents, such as the lipids that carry α-tocopherol (31, 32). The exercise-induced elevation of serum TRAP is probably a reflection of the increased serum α-tocopherol concentration.

In contrast with the findings of the present study, some earlier studies reported that exercise-induced lipid peroxidation in serum may be attenuated by antioxidant supplements in humans (9, 33). The supplementations used in the earlier studies (300 mg vitamin E/d for 4 wk, and 592 mg vitamin E, 1000 mg vitamin C, and 30 mg β-carotene daily for 6 wk) reduced the exercise-induced formation of thiobarbituric acid–reactive material (TBARM). However, the exercise-induced increments were modest (2% in the study by Sumida et al; 9) and the measure used in both studies, TBARM, is notoriously unspecific for lipid peroxidation: prostaglandin endoperoxides, iron, and antioxidants in the sample change the TBA reaction (34–37). Moreover, in the present study, a different antioxidant combination was used.

In conclusion, antioxidant supplementation increased the antioxidant potential of LDL and, to a lesser extent, of serum in healthy male endurance athletes but did not significantly affect the concentration of LDL or serum DC (lipid peroxidation). The finding of increased LDL antioxidant potential supports the idea that α-tocopherol, when coadministered with other antioxidants, is effective in preventing LDL oxidation, but our 4-wk supplementation regimen did not reduce LDL oxidation products. Physical exercise increased serum DC but not LDL-DC, but the increment of serum DC was not affected significantly by antioxidant intervention.

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REFERENCES


