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A marathon run increases the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants

MING-LIN LIU, ROBERT BERGHLOM, SARI MÄKIMATTILA, SANNI LAHDENPERÄ, MIIA VALKONEN, HANNELE HILDEN, HANNELE YKI-JÄRVINEN, AND MARJA-RIITTA TASKINEN

Division of Endocrinology and Diabetology, Department of Medicine, Helsinki University Central Hospital, FIN-00029 HUCH, Helsinki, Finland

Liu, Ming-lin, Robert Bergholm, Sari Mäkimmattila, Sanni Lahdenperä, Miia Valkonen, Hannele Hilden, Hannele Yki-Järvinen, and Marja-Riitta Taskinen. A marathon run increases the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E1083–E1091, 1999.—Physical activity increases the production of oxygen free radicals, which may consume antioxidants and oxidize low-density lipoprotein (LDL). To determine whether this occurs during strenuous aerobic exercise, we studied 11 well-trained runners who participated in the Helsinki City Marathon. Blood samples were collected before, immediately after, and 4 days after the race to determine its effect on circulating antioxidants and LDL oxidizability in vitro. LDL oxidizability was increased as determined from a reduction in the lag time for formation of conjugated dienes both immediately after (180 ± 7 vs. 152 ± 4 min, P < 0.001) and 4 days after (155 ± 7 min, P < 0.001) the race. No significant changes in lipid-soluble antioxidants in LDL or in the peak LDL particle size were observed after the race. Total peroxyl radical trapping antioxidant capacity of plasma (TRAP) and uric acid concentrations were increased after the race, but, except for TRAP, these changes disappeared within 4 days. Plasma thiol concentrations were reduced after the race. No significant changes were observed in plasma ascorbic acid, α-tocopherol, β-carotene, and retinol concentrations after the marathon race. We conclude that strenuous aerobic exercise increases the susceptibility of LDL to oxidation in vitro for up to 4 days. Although the increase in the concentration of plasma TRAP reflects an increase of plasma antioxidant capacity, it seems insufficient to prevent the increased susceptibility of LDL to oxidation in vitro, which was still observed 4 days after the race.

Low-density lipoprotein oxidation; total peroxyl radical trapping antioxidant potential; lipids; low-density lipoprotein size increased production of free radicals. However, if the production of free radicals is excessive, as observed during strenuous aerobic exercise (28, 30), or if antioxidant defenses are severely hampered, the balance between prooxidants and antioxidants is lost. This may lead to tissue damage (20). Thus there is an apparent paradox between the benefits of heavy aerobic exercise on cardiovascular risk factors and the potentially deleterious consequences of free radicals generated during heavy exercise.

Oxidative modification of low-density lipoprotein (LDL) greatly increases its atherogenicity and is considered to be a key step in the development of atherosclerosis (53, 65). The susceptibility of LDL to oxidation in vitro has been reported to be associated with the severity of atherosclerosis (45). Also, autoantibodies against oxidized LDL seem to predict the progression of carotid atherosclerosis (47). Oxidative modification of LDL is induced by oxygen free radicals (39). Generally, LDL in the circulation is well protected against active oxidation by highly efficient plasma antioxidant defense mechanisms. Total peroxyl radical trapping antioxidant capacity of plasma (TRAP) reflects the total combined antioxidant capacity of all individual antioxidants. If, however, oxidative stress exceeds the capacity of the antioxidant defense, LDL may be oxidized.

Marathon running represents an extreme form of physical exercise and provides a model to study the effects of exercise-induced oxidative stress. In the present study, we determined the acute and postexercise effects of a marathon run on the susceptibility of LDL to oxidation in vitro, LDL particle size, antioxidants in LDL and plasma, and TRAP.

SUBJECTS AND METHODS

Subjects. Eleven healthy male marathon runners participating in the Helsinki City Marathon were studied. Written informed consent was obtained after explanation of the purpose, nature, and potential risks of this study to the subjects. The experimental protocol was approved by the Ethical Committee of the Minerva Foundation for Medical Research. Data of marathon runners were compared with those of an age-, sex-, and weight-matched healthy control group (n = 10) of untrained subjects. Clinical characteristics of the study groups are summarized in Table 1.

Study design. The subjects ran a full marathon (42.2 km), except for one subject who interrupted the race after 81 min (16 km). The running times varied from 3.13 to 5.52 h. The mean energy consumption during the race was calculated by multiplying body weight in kilograms by the MET values (metabolic equivalent; work metabolic rate divided by resting metabolic rate; see Ref. 64) and duration of activity in hours

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The oxidation of LDL in vitro was performed by using a modification of the procedure described by Esterbauer et al. (15). The LDL oxidation was initiated by adding freshly prepared CuSO₄ solution to a final concentration of 10.64 µmol/l to the pure LDL solution. The kinetics of LDL oxidation were determined by monitoring the change in absorbance at 234 nm in a motorized six-cuvette cell-equipped Shimadzu spectrophotometer (Shimadzu UV-1201, Shimadzu, Kyoto, Japan) connected to a computer through a RS232 cable. Absorbance was recorded every 2 min. The change in absorbance at 234 nm over time could be divided into three consecutive phases: lag phase, propagation phase, and decomposition phase (15). The lag time (in min), the propagation rate (in mmol · mg LDL⁻¹ · min⁻¹), and the diene concentration (in nmol/mg LDL) were used as measures of LDL to oxidation in vitro.

Measurement of plasma TRAP. TRAP was determined spectrophotometrically using a recently validated method (59). In this assay, we used 2',7'-dichlorofluorescein diacetate (DCFH-DA) to follow the formation of free radicals during decomposition of 2,2'-diazobis-(2-amidinopropane)dihydrochloride (AAPH). Free radicals were formed during thermal decomposition of AAPH in water and followed by measuring the conversion of DCFH-DA to the highly fluorescent dichlorofluorescein (DCF). The DCF formation was measured at 504 nm in a Shimadzu spectrophotometer. Plasma was mixed with PBS to a final dilution of 1%, followed by addition of DCFH-DA to a final concentration of 14 µmol/l. The reaction was started by adding AAPH to a final concentration of 56 mmol/l. The AAPH stock solution was stored at −20°C, thawed, and kept in ice until added to the incubation. Trolox, 8.4 µmol/l, was used as an internal standard, and it was added during the propagation phase when the absorbance had increased to 0.25–0.45. DCF fluorescence or absorbance formation contains four phases. The first lag phase is due to the antioxidants in the sample. After their consumption by free radicals formed from AAPH, the reaction proceeds to the first propagation phase. The second lag phase, which interrupts this propagation, is due to the addition of the internal standard, Trolox, to the incubation, and, in accordance, the second propagation of the reaction follows the consumption of the Trolox. The reaction gives the measured TRAP (TRAPmed).

A theoretical calculated TRAP value (TRAPcalc) can be estimated by using the stoichiometric values for antioxidants after the measurement of their concentrations in human plasma. In the present study, the TRAPcalc was calculated from four main contributors of plasma antioxidant capacity, e.g., tocoferol, ascorbic acid, thiourea acid, and uric acid. The difference between TRAPmed and TRAPcalc is due to antioxidants, which are still unknown, or to the synergism among the various antioxidants present in plasma (TRAPunknown).

Quantitation of LDL particle size. Nondenaturing PAGE was performed on serum samples and was stored at −80°C using gels casted in our laboratory as previously described in detail (34, 40). Gels were stained with Sudan Black B lipid stain and were scanned with a computer-assisted laser scanning densitometer (Personal Densitometer; Molecular Dynamics, Sunnyvale, CA) using a 50-m pixel size and 12-bit signal resolution. Mean particle diameter of the major LDL peak was determined by comparing the mobility of the sample with the mobility of a calibrated reference LDL standard run on each gel. The particle diameters of the reference LDL preparations were determined by electron microscopy. The coefficients of variation for intergel and intragel precisions for the control sample were 0.98 and 1.8%. The cutoff value for large and small LDL particle diameter was set at 25.5 nm, and the size of the mean particle diameter of the major LDL peak was also measured.

Table 1. Clinical characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Marathon Runners</th>
<th>Untrained Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Age, yr</td>
<td>31 ± 3</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178 ± 2</td>
<td>179 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73.6 ± 3.1</td>
<td>80.1 ± 2.3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.3 ± 1.0</td>
<td>24.9 ± 0.6</td>
</tr>
<tr>
<td>Fat, %</td>
<td>11.4 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Maximal aerobic power, ml · kg⁻¹ · min⁻¹</td>
<td>57.2 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>119 ± 4</td>
<td>125 ± 5</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>73 ± 3</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>56 ± 3</td>
<td></td>
</tr>
<tr>
<td>Training history, yr</td>
<td>8.6 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Training extent before marathon, km/wk</td>
<td>46.8 ± 2.1</td>
<td>80.1 ± 2.3</td>
</tr>
<tr>
<td>Best marathon time, min</td>
<td>232 ± 15.6</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as means ± SE.
To avoid interassay variation, the susceptibility of LDL to in vitro oxidation, TRAP, and quantitation of LDL particle size was analyzed in the samples of each subject from different time points (before, immediately after, and 4 days after) at the same run.

Measurement of plasma antioxidants. Plasma α-tocopherol, β-carotene, and retinol were measured by reverse-phase HPLC, as described by Schäfer-Elinder and Walldius (50). We used a Hewlett-Packard reverse-phase HPLC column (ODS Hypersil 5 mm, 200 mm × 2.1 mm) connected to a Waters HPLC system. The latter consisted of a M6000 controller, M486 tunable ultraviolet (UV)-absorbance detector, M717+ autosampler, and Millennium 2010 single-system chromatography manager (Waters, Milford, MA). The UV detector was set at 326 nm for retinol, 292 nm for α-tocopherol, and 450 nm for β-carotene. Plasma ascorbic acid was measured using the spectrophotometric method of Denson and Bowers (8). Plasma protein-bound thiol (sulfhydryl groups) was determined as described by Ellman (11). Plasma uric acid concentrations were measured using a Cobas Mira autoanalyzer (Cobas Mira; Hoffmann-La Roche).

Measurement of lipid-soluble antioxidants in LDL. LDL was isolated by sequential ultracentrifugation (L8–70; Beckman) at the following densities: very-low-density lipoprotein <1.006 g/ml, intermediate-density lipoprotein 1.006–1.019 g/ml, LDL 1.019–1.063 g/ml, high-density lipoprotein (HDL) 1.063–1.210 g/ml.

The concentrations of cholesterol and triglycerides in plasma and lipoprotein subfractions were determined by enzymatic colorimetric assay (Kabi Vitrum, Stockholm, Sweden). The concentrations of total and HDL cholesterol were measured using an autoanalyzer (Cobas Mira; Hoffmann-La Roche).

Maximal aerobic power and body composition. Maximal aerobic power (VO2max) was determined using a work-conducted upright exercise test with an electrically braked cycle ergometer (Bosch ERG 220; Robert Bosch, Berlin, Germany) combined with a continuous analysis of expiratory gases and minute ventilation (Eos-Spint; Erich Jaeger, Wurtzburg, Germany). Exercise was started at a workload of 50 W and was then increased by 50 W at 3-min intervals until perceived exhaustion or until a respiratory quotient of 1.10 was reached. The highest VO2 observed during a 30-s period was defined as VO2max.

Fat-free mass and the percent body fat were determined using bioelectrical impedance plethysmography (Bio-Electrical Impedance Analyzer System, model BIA-101A; RJL Systems, Detroit, MI).

Statistical analyses. Statistical analyses were done using the SYSTAT statistical package (SYSTAT, Evanston, IL). Values are given as means ± SE. The statistical analysis among the repeated observations measured before, immediately after, and 4 days after the race were tested by one-way repeated-measures ANOVA for multiple comparisons. When the overall model proved statistical significance (P < 0.05), the differences between the observations at different time points were tested by Bonferroni t-test. Statistical comparisons between the baseline values before the race in marathon runners and those of sedentary control subjects were performed using the unpaired Student’s t-test. P values < 0.05 were considered as statistically significant. Data are shown as means ± SE.

RESULTS

Susceptibility of LDL to oxidation in vitro and the concentrations of lipid-soluble antioxidants in LDL. The lag time for LDL oxidation was longer in the marathon runners before the race than in the untrained subjects (180 ± 7 vs. 156 ± 10 min, P < 0.05). The lag time, the propagation rate, and diene concentration before and after the marathon run are shown in Table 2. CuSO4-induced LDL oxidation in vitro was accelerated at the early stages of the process, both immediately and 4 days after the race (Fig. 1). As shown in Fig. 2A, the lag time of conjugated diene formation was significantly reduced immediately and 4 days after the race in each of the runners. The rate of oxidation measured as the rate of propagation was also significantly increased immediately after the race, but this change was abolished 4 days after the race. There were no significant differences in the concentration of diene formation during LDL oxidation before or after the race. The concentrations of the lipid-soluble antioxidants in LDL are shown in Table 2. There were no significant changes of α-tocopherol, β-carotene, or retinol concentrations in LDL samples after compared with before the marathon race. The mean size of the major LDL peak was similar immediately and 4 days after the marathon race compared with the baseline value before the race (Table 2).

Table 2. Conjugated diene formation, lipid-soluble antioxidants in LDL, and LDL particle size of subjects before, immediately after, and 4 days after the marathon

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Before</th>
<th>Immediately</th>
<th>4 Days After</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL oxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag time, min</td>
<td>180 ± 7</td>
<td>152 ± 4a</td>
<td>155 ± 7a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diene, nmol/mg LDL</td>
<td>475 ± 20.7</td>
<td>477.3 ± 14.1</td>
<td>487.3 ± 23.9</td>
<td>NS</td>
</tr>
<tr>
<td>Rate, nmol · min⁻¹ · mg⁻¹</td>
<td>3.87 ± 0.24</td>
<td>4.68 ± 0.23</td>
<td>4.31 ± 0.27</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Antioxidants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in LDL, nmol/mg LDL protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol in LDL</td>
<td>10.1 ± 0.9</td>
<td>10.8 ± 1.0</td>
<td>11.0 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>β-Carotene in LDL</td>
<td>0.78 ± 0.10</td>
<td>0.77 ± 0.07</td>
<td>0.73 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Retinol in LDL</td>
<td>0.06 ± 0.00</td>
<td>0.06 ± 0.00</td>
<td>0.063 ± 0.00</td>
<td>NS</td>
</tr>
<tr>
<td>LDL particle size, nm</td>
<td>27.1 ± 0.3</td>
<td>27.5 ± 0.3</td>
<td>27.2 ± 0.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. LDL, low-density lipoprotein; NS, not significant. Statistical comparisons among repeated measures of subjects were tested by one-way ANOVA for repeated measures followed by Bonferroni t-test. aP < 0.001 and bP < 0.05, compared with values before the marathon.
TRAP and circulating antioxidants. TRAP was higher in the marathon runners before the race than in the untrained subjects (1,072 \pm 60 vs. 864 \pm 80 \mu mol/l, P < 0.05). Plasma TRAP and antioxidant data in the runners are shown in Table 3. Immediately after the race, both plasma TRAP$_{\text{mea}}$ and TRAP$_{\text{calc}}$ values corrected for changes in plasma volume (Table 3 and Fig. 3) increased significantly compared with baseline values before the race. The TRAP$_{\text{mea}}$ increased in all subjects (Fig. 2B), and the TRAP$_{\text{calc}}$ increased in all except two subjects (Fig. 2C). After 4 days, TRAP$_{\text{mea}}$ had decreased significantly compared with the values immediately after the race, but it was still significantly and 11.7% higher than the baseline value before the race. TRAP$_{\text{unknown}}$ was increased significantly both immediately and 4 days after the race (Fig. 3).

Immediately after the race, plasma concentrations of uric acid were higher than before the race (Table 3). Plasma thiol concentrations were significantly lower immediately after than before the race (362 \pm 15 vs. 315 \pm 12 \mu mol/l, P < 0.001). Four days after the race, all of these parameters were comparable to the baseline values before the race. We observed no significant changes in plasma ascorbic acid, \(\alpha\)-tocopherol, \(\beta\)-carotene, and retinol concentrations immediately or 4 days after the marathon race. The plasma ascorbic acid (63 \pm 8 \mu mol/l), \(\alpha\)-tocopherol (21.9 \pm 1.7 \mu mol/l), thiols (331 \pm 12 \mu mol/l), and uric acid (336 \pm 12 \mu mol/l) in the untrained subjects were not significantly different from those in the runners.

The extent of training (km/wk) before the marathon correlated positively with the plasma concentration of uric acid (\(r = 0.848, P = 0.001\)) but inversely with the
Table 3. Plasma concentrations of TRAP_{mea}, TRAP_{calc}, ascorbic acid, thiol, uric acid, \( \alpha \)-tocopherol, \( \beta \)-carotene and retinol of subjects before, immediately after, and 4 days after the marathon

<table>
<thead>
<tr>
<th></th>
<th>Before (baseline)</th>
<th>Immediately After (corrected)</th>
<th>4 Days After</th>
<th>One-Way Repeated Measures ANOVA</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAP_{mea}</td>
<td>1.070 ± 0.59</td>
<td>1.338 ± 0.75 ( ^a )</td>
<td>1.197 ± 0.52 ( ^e )</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>TRAP_{calc}</td>
<td>724 ± 20</td>
<td>795 ± 29 ( ^p )</td>
<td>734 ± 17</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>86 ± 10</td>
<td>103 ± 10 ( ^p )</td>
<td>74 ± 4 ( ^e )</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Thiol</td>
<td>362 ± 15</td>
<td>315 ± 12 ( ^a )</td>
<td>346 ± 13 ( ^e )</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>295 ± 16</td>
<td>341 ± 16 ( ^e )</td>
<td>324 ± 15</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Tocopherol</td>
<td>17.5 ± 1.9</td>
<td>19.2 ± 2.1 ( ^e )</td>
<td>17.4 ± 2.1</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>( \beta )-Carotene</td>
<td>0.53 ± 0.04</td>
<td>0.57 ± 0.04 ( ^e )</td>
<td>0.51 ± 0.05</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Retinol</td>
<td>2.13 ± 0.16</td>
<td>2.19 ± 0.19 ( ^e )</td>
<td>2.12 ± 0.15</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Units are \( \mu \)mol/l. TRAP_{mea}, measured trapping antioxidative capacity of plasma (TRAP); TRAP_{calc}, calculated TRAP. Statistical comparisons for repeated observations were tested by one-way ANOVA for repeated measures followed by Bonferroni t-test. Values immediately after the marathon run shown here are results corrected for plasma volume shifts. \( ^a \) gives statistical significance for comparison among baseline, immediately after, and 4 days after repeated values. \( ^p \) < 0.001 before vs. 4 days after, \( ^e \) < 0.05 before vs. 4 days after, \( ^f \) < 0.01 before vs. immediately after. \( ^g \) P < 0.001 before vs. vs. 4 days after. \( ^h \) P < 0.05 immediately after vs. 4 days after.

Fig. 3. Measured (TRAP_{mea}), calculated (TRAP_{calc}), and unknown (TRAP_{unknown}) total peroxyl radical trapping capacity and antioxidant properties of 4 main circulating antioxidants of the marathon runners before, immediately after (corrected for plasma volume shifts), and 4 days after the marathon race. Antioxidant capacities of each antioxidant were calculated by multiplying its plasma concentration by the stoichiometric value (molar amount of free radical trapped by mole of each antioxidant). \( ^* \) P < 0.05, \( ^** \) P < 0.01, and \( ^*** \) P < 0.001 before vs. immediately after. \( ^t \) P < 0.01 and \( ^tt \) P < 0.001, before vs. 4 days after. \( ^i \) P < 0.05 immediately after vs. 4 days after.

plasma concentration of protein thiols before the marathon run \( (r = -0.648, P = 0.031) \).

**DISCUSSION**

In the present study, we found a clear reduction in the lag time of LDL oxidation in vitro when measured immediately and 4 days after a marathon race. The propagation rate of conjugated diene formation also increased significantly. These data indicate that strenuous exercise increases the susceptibility of LDL particle to oxidation in vitro, an effect that is potentially harmful. We also found an increase of plasma TRAP. The increase was explained by an increase in the concentration of the water-soluble antioxidant uric acid and the increase in the unknown component of TRAP, which is thought to reflect synergistic effects of antioxidants and the contribution of unidentified antioxidant mechanisms to TRAP (59, 63).

LDL oxidizability. Sanchez Quesada et al. (48) reported that intensive aerobic exercise increases LDL susceptibility to CuSO_{4}-induced oxidation. Consistent with these data, in the present study, the marathon race increased the susceptibility of LDL particle to oxidation in vitro and decreased the lag time of conjugated diene formation. This change was not a transient phenomenon but persisted over 4 days. Consistent with

Table 4. Lipid parameters of subjects before, immediately after, and 4 days after the marathon

<table>
<thead>
<tr>
<th></th>
<th>Before (baseline)</th>
<th>Immediately After (corrected)</th>
<th>4 Days After</th>
<th>One-Way Repeated Measures ANOVA</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/l</td>
<td>Total</td>
<td>3.96 ± 0.40</td>
<td>4.05 ± 0.43</td>
<td>4.15 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.30 ± 0.06</td>
<td>0.31 ± 0.07</td>
<td>0.22 ± 0.06</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>2.46 ± 0.23</td>
<td>2.49 ± 0.23</td>
<td>2.38 ± 0.20</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>1.52 ± 0.10</td>
<td>1.56 ± 0.12</td>
<td>1.42 ± 0.10</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>Total</td>
<td>0.92 ± 0.14</td>
<td>1.21 ± 0.11</td>
<td>0.99 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.61 ± 0.12</td>
<td>0.66 ± 0.09</td>
<td>0.58 ± 0.15</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein. Statistical comparisons for repeated observations were tested by one-way ANOVA for repeated measures followed by Bonferroni t-test. Values immediately after the marathon run shown here are results corrected for plasma volume shifts. \( ^* \) gives statistical significance for comparison among baseline, corrected immediately after, and 4 days after repeated values. \( ^p \) < 0.05 before vs. 4 days after (a) and immediately after vs. 4 days after (b).
previous cross-sectional data (49), the lag time of LDL to oxidation in marathon runners before the marathon run was longer than in sedentary controls. These data suggest that, while an acute single bout of strenuous exercise increased the susceptibility of LDL to oxidation, other training-associated factors counteract such changes. The degree of LDL oxidation reflects the net effects of its various prooxidants and endogenous antioxidants, the concentration of its oxidizable substrates, especially polyunsaturated fatty acids in LDL (44, 56), and LDL particle size (7). Any alterations of these factors will influence the oxidative modification of LDL and consequently may influence its atherogenicity.

Because oxidation of LDL does not occur until its natural endogenous antioxidants have been consumed (14), the concentration of natural endogenous antioxidants in LDL is an important determinant of the susceptibility of LDL to oxidation. In the present study, the concentrations of lipid-soluble antioxidants (α-tocopherol, β-carotene, and retinol) in LDL were comparable before and after the race. These data are consistent with previous cross-sectional data (49) in which the content of lipid-soluble antioxidants in LDL did not differ between trained and sedentary subjects. Vasan-kari et al. (61) observed no changes in the LDL TRAP during a 31-km run or during a marathon run. Because the concentration of lipid-soluble antioxidants in LDL is the major determinant of the LDL TRAP, these data suggest that acute exercise does not influence the antioxidant defense capacity of LDL particles. Small dense LDL particles are more prone to oxidation than large buoyant particles and are an independent risk predictor of cardiovascular disease (7). In the present study, LDL particle peak size was unaffected by the marathon race. This finding agrees with previous studies in which acute exercise did not significantly change the concentrations of LDL subfractions (2, 48).

Taken together, we cannot explain the observed increase in the susceptibility of LDL to oxidation in vitro by changes in either the concentrations of lipid-soluble antioxidants in LDL or LDL particle size. Of the lipid-soluble antioxidants in LDL, vitamin E seems to be a more effective antioxidant than β-carotene (18, 25, 43). Ubiquinol-10 may, however, protect human LDL even more efficiently against lipid peroxidation than vitamin E (54). Ubiquinol-10 in LDL or plasma was not determined in the present study. Recent studies have reported that HDL can protect LDL from oxidation both in vitro and in vivo (31, 36). Some studies suggest that paraoxonase, an HDL-associated enzyme, may be responsible for the antioxidative action of HDL (37). However, mechanisms via which HDL can protect LDL from oxidation and its contribution to TRAP are still poorly understood. HDL particles seem to be important for transport of circulating plasma lipid hydroperoxides. Mackness et al. (36) reported that inhibition of the formation of LDL lipid peroxides is dependent on the concentration of HDL. It was shown that the increased resistance to oxidation of LDL in trained subjects (compared with sedentary controls) may be due to their increased plasma HDL cholesterol concentration (49).

However, in the present study, the plasma HDL concentrations were similar before and immediately after the marathon run but whether the antioxidant properties of HDL were altered cannot be determined after a marathon run.

Plasma TRAP and circulating antioxidants. In the present study, plasma TRAP was significantly increased both immediately and 4 days after the race. In addition, plasma TRAP was higher in marathon runners before the race than in untrained controls. Vasan-kari et al. (61) have also reported that acute exercise significantly increases serum TRAP. These data suggest that acute exercise activates antioxidant defenses in the body. This activation can be viewed as an adaptive defensive mechanism to cope with increased oxidative stress. Regarding the causes of the increase in TRAP, we found an increase in the concentration of uric acid (Table 3) and in the unidentified antioxidant capacity in plasma (TRAP unkown). (Fig. 3).

During exercise, energy-rich phosphates are utilized, resulting in hypoxanthine accumulation in tissues (52). Conversion of hypoxanthine to uric acid does not occur until an adequate oxygen supply has been reestablished. The latter process is associated with the formation of toxic oxygen free radicals (52). On the other hand, uric acid possesses antioxidant activity (63) and serves as a free radical scavenger in vivo (24). Plasma uric acid can trap peroxy radicals in the aqueous phase and contribute to plasma antioxidant defenses (63). Some previous studies have suggested that acute exercise increases plasma uric acid concentrations (42, 46). This was confirmed in our study in which plasma uric acid increased during exercise, but the change was only temporary and was not seen 4 days after the race. However, we observed a highly significant correlation between the extent of training and the baseline concentration of plasma uric acid before the race. These data suggest that exercise increases uric acid concentration. This increase could reflect enhanced purine oxidation in muscle (46).

Thiols (sulfhydryl groups) are known to scavenge aqueous peroxyl radicals (17, 63). In the present study, the plasma thiol levels were significantly reduced immediately after the marathon run in all subjects (Fig. 2D). These findings are similar to the data reported by Inayama et al. (26), who found acute exercise to decrease plasma thiol concentrations. Wayner et al. (63) reported that plasma thiols are the first antioxidants consumed during 2,2′-azobis(2-amidopropane)hydrochloride-initiated peroxidation of plasma. Thus the observed decrease of the plasma thiol concentrations could reflect their oxidation by exercise-induced free radicals in the aqueous phase of plasma. Physical training may also chronically deplete plasma thiols, since we found an inverse correlation between the extent of training and baseline plasma thiol concentrations.

The effect of acute exercise on plasma ascorbic acid concentrations has been variable. Both increases (46, 62) and no change (19) in plasma ascorbic acid concentrations have been reported in different studies. In the
present study, the plasma ascorbic acid concentration tended to increase after the race, but the change was not significant. Some (46, 61), but not all (38, 62) previous studies, have suggested that exercise increases plasma \( \alpha \)-tocopherol concentrations. The increase of plasma \( \alpha \)-tocopherol induced by exercise may be due to a shift in the interorgan distribution where liver and adipose tissue may be donors and muscles and heart are vitamin E receivers (58). In our study, immediately after the race, the plasma \( \alpha \)-tocopherol concentration, corrected for the plasma volume shifts, was not significantly increased. Several other circulating plasma antioxidants and antioxidant enzymes (\( \beta \)-carotene, retinol, ubiquinol-10, bilirubin, glutathione, glutathione peroxidase, catalase, superoxide dismutase, transferin, and ceruloplasmin) also contribute to the plasma antioxidant capacity (21, 27–30, 46, 51, 62, 63). The relative contribution of these components to TRAP is, however, trivial (63). In the present study, exercise had no effect on the plasma \( \beta \)-carotene and retinol concentrations. These findings are consistent with previous studies in which acute exercise did not change plasma \( \beta \)-carotene (46) and retinol levels (46, 61).

Plasma antioxidants can also act synergistically in vivo to provide more protection against free radical damage than could be provided by any single antioxidant alone (63). In the present study, before the race, TRAP\(_{\text{calc}}\) and TRAP\(_{\text{unknown}}\) amounted to 68 and 32% of the TRAP\(_{\text{max}}\) (Fig. 3). In contrast, after the marathon run, the increase of TRAP\(_{\text{max}}\) was due to a 73% increase of TRAP\(_{\text{unknown}}\), whereas the increases in the concentrations of uric acid and other antioxidants accounted for 27%. This marked increase of TRAP\(_{\text{unknown}}\) suggests that acute exercise may activate unidentified antioxidant defenses as a physiological adaptive response against oxidative stress. Even so, neither of the components of TRAP seem sufficient to prevent the increased susceptibility of isolated LDL particle to oxidation in vitro.

Lipid and lipoproteins. Previous data (10) have shown that regular physical training is associated with lowering of plasma total triglycerides, total cholesterol and LDL concentrations, and an increase in the plasma HDL cholesterol concentration. The effect of a single bout of exercise on lipid concentrations has, however, been highly variable (10, 41). For example, increases (9), decreases (57), or no change (33) in plasma total triglycerides and/or total cholesterol have been reported in different studies. The changes of plasma LDL cholesterol concentrations have also been variable, whereas the increase in HDL cholesterol has been more consistent (9, 33, 57). In the present study, the concentrations of total cholesterol, triglycerides, and LDL cholesterol remained unchanged immediately and 4 days after the marathon run. The plasma HDL cholesterol concentration immediately after the race was slightly, but not significantly, increased compared with the baseline value.

Conclusion. A single bout of heavy endurance physical activity increases TRAP and some (uric acid, TRAP\(_{\text{unknown}}\)) of its components. These increases can be viewed as an adaptive defensive mechanism against exercise-induced oxidative stress. The decrease of the plasma thiol concentration immediately after the race may reflect its role as a first-line defense mechanism and consumption by reactive oxygen species in the aqueous phase of plasma. The marathon run also increased the susceptibility of LDL to oxidation in vitro, and this change persisted over 4 days. This increased susceptibility of LDL to oxidation in vitro was not explained by changes in either endogenous lipid-soluble antioxidants in LDL or LDL particle size.

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Address for correspondence: M.-R. Taskinen, Div. of Endocrinology and Diabetology, Dept. of Medicine, Haartmaninkatu 4, PO Box 340, FIN-00029 HUCH, Helsinki, Finland (E-mail: mataskin@helsinki.fi).

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