Exercise performance, red blood cell deformability, and lipid peroxidation: effects of fish oil and vitamin E


Exercise performance, red blood cell deformability, and lipid peroxidation: effects of fish oil and vitamin E. J. Appl. Physiol. 83(3): 746–752, 1997.—Previous studies have indicated that fish oil supplementation increases red blood cell (RBC) deformability, which may improve exercise performance. Exercise alone, or in combination with an increase in fatty acid unsaturation, however, may enhance lipid peroxidation. Effects of a bicycle time trial of ~1 h on RBC characteristics and lipid peroxidation were therefore, studied in 24 trained cyclists. After 3 wk of fish oil supplementation (6 g/day), without or with vitamin E (300 IU/day), trial performance, RBC characteristics, and lipid peroxidation were measured again. RBC deformability appeared to decrease during endurance exercise. After correction for hemoconcentration, plasma total tocopherol concentrations decreased by 0.77 µmol/l (P = 0.012) or 2.9% and carotenoid concentrations by 0.08 µmol/l (P = 0.0008) or 4.5%. Endurance exercise did not affect the lag time and rate of in vitro oxidation of low-density lipoproteins (LDLs), but the maximum amount of conjugated dienes formed decreased by 2.1 ± 1.0 µmol/mmol LDL cholesterol (P = 0.042) or 1.2%. Fish oil supplementation with and without vitamin E did not affect RBC characteristics or exercise performance. Both supplements decreased the rate of LDL oxidation, and fish oil supplementation with vitamin E delayed oxidation. The amount of dienes, however, was not affected. The supplements also did not change effects of exercise. We conclude that the changes observed during endurance exercise may indicate increased oxidative stress, but further research is necessary to confirm this. Fish oil supplementation does not improve endurance performance, but it also does not cause or augment changes in antioxidant levels or LDL oxidation during exercise.

However, incorporation of the highly unsaturated fatty acids in membranes may increase the membranes’ susceptibility to lipid peroxidation, especially in combination with exercise. Previously, we (21) have reported that the susceptibility to oxidation of low-density lipoproteins (LDLs) was increased by 20% after fish oil supplementation and that this potentially unbeneficial effect could be counteracted by the antioxidant vitamin E.

Therefore, we have investigated the effects of exercise and fish oil supplementation, with or without vitamin E, on the deformability of RBCs and lipid peroxidation and the effects of the supplements on exercise performance.

METHODS

Subjects and Study Design

Twenty-four apparently healthy, well-trained male cyclists (aged 19–42 yr) entered the study between January 11 and 26, 1995. Written informed consent was received from each subject, and prior approval for the study was obtained from the Medical Ethics Committee of Maastricht University. Participants were asked to abstain from intense physical activity for 36 h before the measurements and were instructed not to use any antioxidant supplements during and 2 wk preceding the study. At the start of the study, maximal workload capacity (Wmax) of each participant was determined. Four to 7 days later, the subjects performed an endurance exercise test on a cycle ergometer. For the next 3 wk, eight subjects received a placebo supplement (P group), eight subjects received a fish oil supplement (F group; 6 g of fish oil daily), and eight subjects received the fish oil supplement together with vitamin E (FE group; Ephynal 300, 300 IU dl-α-tocopherol acetate daily). Data from previous studies (11, 12, 14, 21, 29) indicated that eight subjects in each group were sufficient to detect, with a power of 80%, a difference in change between the experimental groups for RBC deformability of <2%, for endurance performance of 7%, and for LDL oxidation in vitro of 10%. The three groups were stratified for Wmax, and, as far as possible, one subject from each group was tested on the same day. At the end of the study, exercise testing procedures were repeated.

Placebo capsules, containing microcrystalline cellulose, were supplied by the pharmacy of the Academic Hospital Maastricht (Maastricht, The Netherlands). The fish oil capsules (Fish-EPA) were a generous gift from Orthica (Weesp, The Netherlands), and Ephynal 300 capsules were from Hoffmann-La Roche (Basel, Switzerland). The fish oil capsules contained (wt/wt) 17.6% eicosapentaenoic acid (EPA) C20:5 (n-3) and 12.5% docosahexaenoic acid (DHA) C22:6 (n-3), and only 0.12% vitamin E (0.01% δ-tocopherol, 0.03% β+γ-tocopherol, 0.08% α-tocopherol), as analyzed by gas chromatography and high-performance liquid chromatography (HPLC), respectively.

THE DEFORMABILITY OF RED BLOOD CELLS (RBCs) decreases during exercise (8, 29), which may impede blood flow through various regions of the microcirculation (23). The reduced deformability may, at least partly, be due to increased production of free radicals during exercise, which induces peroxidation of unsaturated fatty acids in membrane phospholipids and polymerization of membrane proteins (26). Several studies have also indicated that dietary fish oils increase the deformability of RBC as a result of the incorporation of (n-3) fatty acids in the membrane (4, 28) and facilitate the transport of RBC through the microcirculation (3). Therefore, fish oil supplementation may enhance oxygen supply to muscles and improve exercise performance.
Exercise Performance Protocols

Wmax test. Wmax was determined during an incremental cycle ergometer test with 3-min intervals (16). Oxygen uptake was measured continuously (Oxycon Delta, Mijnhard, The Netherlands). Wmax of each subject, as measured the week before supplementation started, was used for both endurance exercise tests. The second Wmax test, performed after 15–17 days of supplementation, was used to detect changes in Wmax and plasma lactate concentrations due to the supplements.

Endurance exercise test. Before and after the 3-wk period of supplementation, subjects were asked to perform a prefixed absolute workload on a linearly functioning cycle ergometer (Lode Excalibur Sport, Lode, Groningen, The Netherlands), as little time as possible. The workload was based on 70% of the Wmax of each subject, and the workload output depended directly on the pedaling frequency. During the test, subjects were only informed about the cumulative achieved amount of work, as displayed by a 0–100% indicator of the total workload. Time as well as pedaling frequency were blinded to the subjects. Subjects were allowed to drink tap water ad libitum. The time needed to complete the test was used as a measure of physical performance. This new validated endurance exercise test, resembling a time trial of 1 h, has been described in detail by Eijckendrup et al. (14).

Blood Sampling

\( \dot{V}_{\text{max}} \) test. At the end of each workload step, antecubital blood was collected via an intravenous Teflon catheter (Baxter, Utrecht, The Netherlands) in Na2EDTA·2H2O (final concentration 2 g/l). Samples were centrifuged for 10 min at 1,600 g and 4°C, and plasma was stored at –40°C for lactate analysis later.

Endurance exercise test. Before and immediately after each test, fasting free-flowing antecubital venous blood was collected in decapped K3EDTA-containing Monoject tubes (final concentration 1.5 g/l; Sherwood Medical, Ballymoney, Northern Ireland) and in heparin (final concentration 143 US Pharmacopea units/5 ml; Monoject tubes). Tubes were immediately closed, and blood and anticoagulant were carefully mixed. One tube with EDTA-anticoagulated blood was sent to Amsterdam (at ambient temperature) and was analyzed the week of supplementation, was used to detect changes in Wmax and plasma lactate concentrations due to the supplements.

Blood Analyses

Lactate. EDTA-containing plasma, collected during Wmax tests, was analyzed for lactate (enzymatic lactate dehydrogenase method; L DH no. 106984, Boehringer Mannheim, Mannheim, Germany) on a COBAS-BIO centrifugal analyzer (Hoffmann-La Roche).

Hematologic variables. RBC deformability, and plasma viscosity. Hematologic variables in EDTA-anticoagulated blood were analyzed on a Coulter counter (model MD 18, Miami, FL), and changes in blood and plasma volume during endurance exercise were calculated by using hematocrit (Hct) and hemoglobin (Hb) values (5).

RBC deformability was measured based on the ektacytometric principle using a laser-assisted optical rotational cell analyzer ([LORCA] R&R Mechatronics, Hoorn, The Netherlands) (11). Briefly, 25 µl of EDTA-anticoagulated blood were suspended in 5 ml of a 5% solution of polyvinylpyrrolidone (300 mosmol/l, Sigma Chemical, St. Louis, MO). This sample was then subjected to several rotational speeds at 21°C, giving final shear stresses ranging from 0.30 to 53.35 Pa. Laser diffraction was used to follow the change of the RBC population from biconcave toward an ellipsoid morphology under increased shear stress, and the deformability (elongation index [EI]) of the RBCs was calculated from the major and minor axes of the ellipsoid diffraction pattern (Fig. 1). Apart from the near-maximum values at a shear stress of 30 Pa as previously reported (29), we also evaluated the EI at 0.95, 3, and 9.5 Pa, because, for example, rigidification of RBC with glutaraldehyde decreases the EI only at lower shear stresses (12). The rest of the EDTA-containing blood was centrifuged for 10 min at 3,000 g and room temperature, and the viscosity of the plasma was measured in duplicate at a shear rate of 70/s by using a Contraves LS 30 viscometer (Contraves, Zürich, Switzerland). Phospholipid fatty acids. Phospholipids were extracted from the RBCs, and their fatty acids were methyalted and analyzed by gas chromatography as described by Foreman-van Drongelen et al. (7), with the modification that fatty acids were separated on BPX70 polar and BP1 nonpolar capillary columns (Scientific Glass Engineering, Austin, TX). Fish oil from the capsules was directly methylated with triheptadecanoin as an internal standard. The polyunsaturated fatty acid (PUFA) unsaturation index was calculated as the sum of the molar percentage of each PUFA (mol/mol total fatty acids·100%) multiplied by its number of double bonds.

Plasma antioxidants. EDTA-containing plasma was analyzed for retinol, tocopherols, and carotenoids by HPLC with simultaneous absorbance and fluorescence detection as described by Hess et al. (13), with the modification that an internal standard (retinol acetate) was used, and extraction of lipids was performed twice to improve recovery. Fish oil capsules were dissolved (20 mg oil/ml) in ethanol-dioxane-acetonitrile (1:1:3, EDA) and were analyzed by HPLC. Chromatogram peak areas were calibrated against standards dissolved in EDA. Because pure phytofluene was not available, quantitative determination of this compound was not possible. β-Tocopherol coeluted with γ-tocopherol. The canthaxanthin standard eluted separately from the lutein standard, but in plasma samples canthaxanthin appeared as a shoulder.

Fig. 1. Change in diffraction pattern during elongation of red blood cells exposed to various shear stresses in vitro (log scale). Elongation index (EI) is calculated from major (A) and minor (B) axes of ellipsoid diffraction pattern.
RESULTS

Effects of Endurance Exercise Before Supplementation

Body weights were similar in all groups (P: 74.8 ± 8.0; F: 74.5 ± 6.3; FE: 71.6 ± 6.1 kg) throughout the study. During the presupplementation endurance exercise test, subjects lost on average 0.56 ± 0.07 kg (P ≤ 0.0001) of body weight and consumed on average 0.65 ± 0.06 liter of water.

Effect of endurance exercise on hematologic variables, RBC deformability, and plasma viscosity. During the presupplementation endurance exercise test, the average RBC concentration, total Hb, and Hct increased by 5.3–5.7%. White blood cell concentrations increased from 5.38 ± 0.16 × 10^9/l before exercise to 7.48 ± 0.29 × 10^9/l after exercise (P ≤ 0.0001). Platelet concentrations increased from 202 ± 7 × 10^9/l to 259 ± 10 × 10^9/l (P ≤ 0.0001). Blood volume decreased by 5.0 ± 0.6% during exercise and plasma volume by 9.0 ± 1.0%. RBC deformability decreased during exercise, but only the decrease in EI of 0.005 ± 0.002 (P = 0.035) or 1.6% at a shear rate of 3 Pa reached statistical significance. Plasma viscosity increased from 1.46 ± 0.02 mPa·s before exercise to 1.52 ± 0.02 mPa·s after exercise (P = 0.005).

Effect of endurance exercise on plasma antioxidants. Unadjusted plasma antioxidant concentrations increased during endurance exercise. After correction for the decrease in plasma volume in each subject, however, plasma antioxidant concentrations decreased significantly, except for retinol (Table 1). Total tocopherol concentrations decreased by 0.77 ± 0.28 µmol/l (P = 0.012) or 2.9 ± 1.1%, and total carotenoid concentrations decreased by 0.08 ± 0.02 µmol/l (P = 0.0008) or 4.5 ± 1.2%.

Effect of enduranc exercise on LDL oxidation in vitro. The lag time before onset of rapid oxidation of LDL in vitro, the maximum rate of oxidation, and the TRmax were not affected by endurance exercise. The maximum amount of conjugated dienes formed in vitro decreased by 2.1 ± 1.0 µmol/mmoll LDL cholesterol (P = 0.042) during the presupplementation endurance exercise test (Table 2).

Effects of Fish Oil and Vitamin E Supplementation

Effect of supplements on RBC phospholipid fatty acids. Compliance with the fish oil supplements was confirmed by an increase in the concentration of (n-3) PUFAs in RBCs from 5.2 to 7.0 g/100 g of fatty acid in the F group (P ≤ 0.0001 vs. P) and from 5.2 to 7.6 g/100 g in the FE group (P ≤ 0.0001 vs. P) during the study (Table 3). The increase in EPA was larger in the FE group (P = 0.001 vs. F), but other indicators of compliance, such as changes in DHA and intake of capsules, were similar. Although increases in (n-3) PUFAs were accompanied by decreases in (n-6) PUFAs, the degree of PUFA unsaturation was significantly increased after supplementation with fish oil (Table 3).

Effect of supplements on Wmax. Prestratification by Wmax at the start of the study resulted in similar values of 5.0 ± 0.2, 4.9 ± 0.2, and 5.0 ± 0.2 W/kg in the P, F, and FE groups, respectively. During the study,
exercise, were similar in all groups and changed by only 0.11 (6) between the groups (6 the F group, and by 0.15 (6 groups. After supplementation, V˙O2max increased by 10.220.33.6 on June 8, 2017 http://jap.physiology.org/ Downloaded from
1.93 ± 0.25 to 0.75 ± 0.09 µmol/l (P < 0.004 vs. other groups).

Decreases in plasma antioxidant concentrations during endurance exercise were smaller after fish oil and fish oil and vitamin E supplementation than were exercise-induced decreases after P supplementation (Table 1). However, differences between the groups did not reach statistical significance.

Effect of supplements on LDL oxidation in vitro. Before supplementation, LDL oxidation parameters were similar in all three groups. At the end of the supplementation period, the lag time tended to increase in the FE group compared with the other groups (P = 0.052 vs. F; P = 0.113 vs. P; Fig. 2). The rate of oxidation in vitro decreased by 0.52 ± 0.16 µmol dienes·mmol LDL cholesterol−1·min−1 in the F group (P = 0.010 vs. P) and by 0.82 ± 0.18 µmol dienes·mmol LDL cholesterol−1·min−1 in the FE group (P = 0.0003 vs. P; P = 0.159 vs. F), whereas the P group showed a slight increase (0.07 ± 0.08 µmol dienes·mmol LDL cholesterol−1·min−1); Fig. 2). TRmax remained unchanged in the P and F groups but increased significantly in the FE group (change: 15.0 ± 5.7 min; P = 0.007 vs. P; P = 0.040 vs. F; Fig. 2). The maximum amount of dienes formed in vitro did not change significantly during the study, and no differences were, therefore, noticed between the groups (Fig. 2).

DISCUSSION

The present study examined the effects of fish oil and vitamin E supplementation on exercise performance, RBC deformability, plasma antioxidant status, and in vitro LDL oxidation.

Effects of Endurance Exercise Before Supplementation

Effect of endurance exercise on hematologic variables, RBC deformability, and plasma viscosity. During endurance exercise, hemococoncentration of ~5% was observed, which was caused by a 9% reduction of the plasma compartment. At the same time, RBC deformability decreased at 3 Pa but not at 30 Pa, as reported by Van der Brug et al. (29) after their subjects performed a combination of cycling and running for 140 min. After a marathon, whole blood filterability was also impaired (8). Others, however, did not find a decrease in RBC filterability after a marathon (20). Guezennec et al. (9) observed a decrease in RBC filterability during a 1-h cycling exercise at a simulated altitude of 3,000 m in a hypobaric chamber but not at sea level. Although a decrease in RBC deformability is usually reported after longer exercises, the sensitive

Table 3. Fatty acid composition of fish oil concentrate and effects of fish oil supplementation on fatty acid composition of red blood cell phospholipids

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Fish oil</th>
<th>Fish oil + vitamin E</th>
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</thead>
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<tr>
<td><strong>Red Blood Cell Phospholipid Fatty Acids in Experimental Groups</strong></td>
<td><strong>Baseline</strong></td>
<td><strong>Change</strong></td>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td><strong>Total saturates</strong></td>
<td>25.9</td>
<td>47.9 ± 0.3</td>
<td>47.4 ± 0.2</td>
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<tr>
<td><strong>Total monenes</strong></td>
<td>26.5</td>
<td>17.8 ± 0.2</td>
<td>18.2 ± 0.4</td>
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<tr>
<td><strong>Total polyenes</strong></td>
<td>39.7</td>
<td>33.5 ± 0.2</td>
<td>33.4 ± 0.4</td>
</tr>
<tr>
<td><strong>(n-6) Polyenes</strong></td>
<td>3.8</td>
<td>28.4 ± 0.2</td>
<td>28.0 ± 0.4</td>
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<tr>
<td><strong>C18:2 (n-6)</strong></td>
<td>1.2</td>
<td>12.4 ± 0.4</td>
<td>12.3 ± 0.3</td>
</tr>
<tr>
<td><strong>C20:4 (n-6)</strong></td>
<td>1.1</td>
<td>10.7 ± 0.2</td>
<td>10.4 ± 0.2</td>
</tr>
<tr>
<td><strong>(n-3) Polyenes</strong></td>
<td>35.7</td>
<td>4.9 ± 0.2</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td><strong>C20:5 (n-3)</strong></td>
<td>17.6</td>
<td>0.5 ± 0.1</td>
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</tr>
<tr>
<td><strong>C22:6 (n-3)</strong></td>
<td>12.5</td>
<td>2.4 ± 0.0</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td><strong>Unidentified</strong></td>
<td>7.9</td>
<td>10.0 ± 0.1</td>
<td>10.0 ± 0.1</td>
</tr>
<tr>
<td><strong>Polyene unsaturation index</strong></td>
<td>102.1 ± 0.9</td>
<td>9.0 ± 0.5</td>
<td>102.4 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE given in g/100 g fatty acids for 24 subjects. Subjects received a placebo, fish oil (6 g/day), or fish oil (6 g/day) plus vitamin E (300 IU/day) supplement to their habitual diets for 3 wk. *Calculated as sum of percentage of each polyene (mol/mol total fatty acids·100%) multiplied with its no. of double bonds. Changes after supplementation in 3 groups were compared by analysis of variance: #significant difference from change in placebo group, †P < 0.01; ‡P = 0.0001; §significant difference from change in fish oil group, P < 0.01.
and reproducible LORCA technique (12) used in this study also detected a decrease after a 1-h trial.

In the present study, the change in RBC deformability was small (~2%), but together with the 4.2% increase in plasma viscosity it may have a significant effect on whole blood viscosity, as previously demonstrated (29).

Effect of endurance exercise on plasma antioxidants. Plasma tocopherol and carotenoid concentrations, adjusted for hemoconcentration, decreased during exercise. Indirectly, this may indicate increased lipid peroxidation. After 45 min of running (18) or 90 min of submaximal cycling (30), no changes in plasma vitamin E were reported. Data from the latter study, however, were not adjusted for hemoconcentration, and a decrease in plasma vitamin E is, therefore, conceivable. Measurements of blood reduced and oxidized glutathione after submaximal cycling also indicated increased oxidative stress, but this was insufficient to cause damage to RNA (30).

On the other hand, another explanation for the decreased antioxidant concentrations is possible. Exercise-activated lipoprotein lipase (27) may have caused a transfer of tocopherols (15), and maybe also of carotenoids, from the circulation to tissues. This might also explain why plasma concentrations of retinol, which is mainly transported via a specific retinol binding protein, and much less via lipoproteins, did not decrease during exercise. However, others showed that, in muscle tissue, vitamin E concentrations also decrease after exercise (18).

Effect of endurance exercise on LDL oxidation in vitro. LDL oxidation in vitro was little affected by endurance exercise. The amount of conjugated dienes formed in vitro decreased slightly after exercise, and theoretically this indicates that the relative amounts of PUFAs in the native LDLs had decreased (22). This agrees with results of Sumikawa et al. (24), who showed that exercise decreased the proportion of PUFAs in RBC phospholipids of untrained subjects.

Effects of Fish Oil and Vitamin E Supplementation

Effect of supplements on exercise performance. Exercise performance, as measured by validated exercise tests, was not significantly altered by fish oil or fish oil and vitamin E supplementation in the present study. The Wmax slightly increased during the study in all groups. This small increase, however, was insufficient to improve endurance performance during the time trial. Nor were effects of fish oil supplementation on Wmax or VO2max found in sedentary subjects (1). In well-trained subjects, Leaf and Rauch (17) found an increase in VO2max (predicted VO2max, based on treadmill performance) after daily supplementation with 6 g of fish oil, but no increase in VO2max was noticed after supplementation with 12 g/day, and neither supplement improved treadmill performance.

Effect of supplements on RBC deformability. The ~2 g/100 g increase in (n-3) PUFAs in membrane phospholipids of RBC after fish oil supplementation did not affect RBC deformability. Results from other studies are conflicting. Earlier studies showed that supplementation with ~3.5 g/day of EPA (4) or fish oil (28) for 4–6 wk increases RBC (4) and whole blood (28) filterability, but no control groups were used. Guezennec et al. (9) reported that in trained subjects compared with nonsupplemented trained controls, daily supplementation with 2 g of fish oil for 6 wk improved filterability of washed RBCs after hypoxic exercise. Preexercise RBC filterability, however, was not improved by the supplement (9). A dose-response study also showed no effect of supplementation with up to 6 g/day of (n-3) fatty acid ethyl ester for 12 wk on filterability of 10% RBC solutions (2). The latter studies, together with the present one, show that supplementation with fish oil in moderate quantities does not significantly affect RBC deformability. Although supplementation with large quantities might affect RBC deformability, this is probably not feasible in humans.

Effect of supplements on plasma antioxidants. Fish oil supplementation did not affect plasma antioxidant concentrations. A decrease in plasma antioxidant concentrations after fish oil supplementation has been reported (19), but this was not confirmed in the present study or by others (10). Additional vitamin E supplementation, however, increased plasma α-tocopherol and decreased plasma β+γ-tocopherol concentrations, as has been found by others (18).

Effect of supplements on LDL oxidation in vitro. The lag time during oxidation of LDL in vitro was not affected by fish oil supplementation and was only nonsignificantly increased by additional vitamin E supplementation. A previous study in sedentary male subjects (21) showed a larger increase in lag time after fish oil and vitamin E supplementation compared with a decrease in lag time in subjects who received fish oil supplementation without vitamin E. Recently, Suzukawa et al. (25) also found a decrease in lag time after fish oil supplementation with corn oil supplementation in drug-treated hypertensive subjects.

In the present study, both fish oil and fish oil and vitamin E supplementation reduced the rate of LDL oxidation in vitro. This not only was found in a previous study (21) but also was reported by Suzukawa et al. (25). The mechanism responsible for this decrease is unknown, however. The decrease in rate of oxidation in the F group hardly affected the TRmax. Additional vitamin E supplementation delayed oxidation of LDL, although the apparent difference with the F group was only of borderline significance.

Fish oil supplementation did not affect the amount of conjugated dienes formed during LDL oxidation in vitro, whereas a previous study (21) showed a 20% increase after supplementation with a similar amount of fish oil. In addition, the in vitro formation of thiobarbituric acid-reactive substances in LDL samples is also increased after fish oil supplementation (10, 25). Although the fast method of LDL preparation used in the present study was different from the time-consuming method in the previous one (21), comparison of the two methods in sedentary volunteers showed no significant difference in the amount of conjugated dienes formed in vitro (results not reported).
In conclusion, this study shows that a 1-h intense exercise decreases RBC deformability, causes consumption or a shift of plasma tocopherols and carotenoids, and decreases the formation of conjugated dienes during oxidation of LDL in vitro. Although these changes may suggest increased oxidative stress during exercise, the physiological meaning of these small changes need further investigation. Contrary to previous findings, no adverse effects of fish oil supplementation on parameters of lipid peroxidation were found. However, this study also shows that moderate fish oil supplementation does not significantly improve RBC deformability or physical performance.

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Address for reprint requests: R. P. Mensink, Maastricht Univ., Dept. of Human Biology, PO Box 616, 6200 MD Maastricht, The Netherlands.

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