The Redox Status of Coenzyme Q10 in Total LDL as an Indicator of In Vivo Oxidative Modification

Studies on Subjects With Familial Combined Hyperlipidemia

Yolanda B. de Rijke, Sebastian J.H. Bredie, Pierre N.M. Demacker, Janine M. Vogelaar, Heidi L.M. Hak-Lemmers, Anton F.H. Stalenhoef

Abstract Familial combined hyperlipidemia (FCH) is characterized by a familial occurrence of a multiple-type hyperlipidemia, associated with coronary risk. The latter may be related to increased levels of small, dense LDL particles that have been found to be more prone to oxidative modification. We isolated total LDL as fresh as possible from 12 normolipidemic relatives with a buoyant LDL subfraction profile (group 1), 7 normolipidemic subjects with a dense LDL subfraction profile (group 2), and 16 hyperlipidemic FCH subjects with a dense LDL subfraction profile (group 3). In these nonobese and normotensive men, we studied the resistance of total LDL against Cu2+-oxidation in vitro. In addition, we analyzed the α-tocopherol and the coenzyme Q10 contents of LDL and determined their relation to LDL oxidizability. LDL isolated from group 2 subjects was more susceptible to oxidative modification than LDL from group 1 subjects (lag time: 60.4±8.1 versus 70.4±11.4 minutes; P<.05). For the combined groups, the ratio of ubiquinol-10 to polyunsaturated fatty acids in LDL, together with the basal amount of dienes in LDL, were good predictors of the rate of LDL oxidation (R2=.73, P=.0001). In groups 2 and 3, the redox status of coenzyme Q10 (ubiquinol-10/ubiquinone-10) and the ratio of ubiquinol-10 to α-tocopherol in LDL were reduced compared with group 1 (P<.05). The K-value, a measure of the LDL density, correlated with the redox status (r=.37, P<.05). We conclude that in subjects with FCH total LDL is more prone to oxidation, due to the predominance of dense LDL particles. In addition, the decreased redox status of coenzyme Q10 in LDL from subjects with a dense LDL subfraction profile suggests that the LDL in the circulation has already undergone some oxidation. 

Key Words • LDL subfraction profile • antioxidants • α-tocopherol • ubiquinol-10 • ubiquinone-10 • lipid peroxidation

Familial combined hyperlipidemia (FCH), first identified by Goldstein et al., is a genetically and metabolically heterogeneous common lipid disorder, associated with an increased risk for cardiovascular disease. The trait is characterized by a multiple-type hyperlipidemia in first-degree relatives. Affected persons exhibit elevated plasma concentrations of cholesterol or triglycerides, or both. In addition, elevated concentrations of apolipoprotein B and reduced concentrations of HDL-C are observed. Furthermore, FCH has been shown to be associated with a predominance of dense LDL subfraction profiles. Small, dense LDL was generally found to be prevalent in patients with coronary heart disease. Studies on LDL oxidation revealed that small, dense LDL particles are more prone to oxidative modification than buoyant LDL. Oxidation of LDL is believed to play an important role in early atherosclerosis. After being oxidatively modified in the intima, LDL is probably taken up by scavenger receptors of macrophages. As a result of uncontrolled uptake of oxidized LDL, macrophages are converted into cholesterol-rich foam cells, a hallmark of early atherosclerotic lesions. Evidence in humans to support the oxidation hypothesis has been provided by studies that showed an association between plasma autoantibodies against oxidatively modified LDL and the progression of cardiovascular disease.

According to the oxidation hypothesis, LDL is protected against oxidative stress by antioxidants, thereby delaying the formation of modified LDL. The lipophilic antioxidants α-TOH, QH2-10, β-carotene, and lycopene are present in LDL. Lipid epidemiological studies suggested that high (>100 mg/d) α-TOH intake contributes to reduced risk of atherosclerosis. Although α-TOH is believed to be the major antioxidant in LDL responsible for the protection against oxidation in vivo, extensive work by Stocker and co-workers (reviewed in Reference 23) suggests that the determination of the LDL QH2-10 content is more relevant to assess the initial state of LDL peroxidation in vivo. QH2-10 (or reduced coenzyme Q) is an endogenous product of the mevanolate pathway, having coenzymatic activity in the enzyme system of mitochondria, where it functions as an essential electron carrier in the respiratory system. This antioxidant is also present in foods such as soybeans, walnuts, almonds, oils, fruits, and spinach. Despite low concentrations in LDL (0.5 to 0.8 mol/LDL particle), compared with LDL...
alpha-Tocopherol content (8 to 15 mol/LDL particle), QH2-10 has shown itself to be the first-line defense against oxidative stress in LDL.26 In volunteers, a long-term supplementation with Q10 resulted in a twofold increase in the plasma concentration of QH2-10.27 This was accompanied by increased resistance of LDL against oxidation, initiated by aqueous peroxyl radicals, in vitro.27 Besides the contribution of antioxidants, LDL oxidizability in vitro was found to be influenced by the fatty acid composition28 and the chemical composition of LDL.11

Although a specific marker to definitively ascertain the diagnosis of FCH is still lacking, a recent study shows that a predominance of small, dense LDL particles is characteristic for affected FCH relatives.4 especially when accompanied by hyperlipidemia. Regarding the observations on the relative constancy of the LDL subfraction profile in relation to that of plasma lipid concentrations, especially that of plasma triglycerides (S.H. Bredie, P.N.M. Demacker, A.F.H. Stalenhoef, unpublished observations, 1995), we assume that the presence of dense LDL is a helpful metabolic marker for identifying affected relatives of FCH kindreds characterized by premature cardiovascular disease.4 Premature cardiovascular disease is a hallmark of FCH.4

Methods

Chemicals
Q10, dl-alpha-TOH, and BHT were from Sigma Chemical Co; Q10 was reduced to QH2-10, essentially as described by Frei et al.29 All reagents and HPLC solvents were of high analytical grade.

Subject Selection
All participants were selected from 40 well-defined FCH families, consisting of both affected and nonaffected relatives.4 Diagnosis of FCH was based on the following criteria: (1) the presence in first-degree relatives of a multiple-type hyperlipidemia with elevated levels of total plasma cholesterol and/or triglycerides using the age- and sex-related 90th percentile upper levels of the prospective cardiovascular Münster (PROCAM) study30 and (2) a family history of premature cardiovascular disease before age 60. Families were excluded when first-degree family members had tendon xanthomata. None of the probands was homozygous for the apo E2 allele, and for all probands, a secondary cause (e.g., diabetes mellitus, hypothyroidism, and hepatic or renal impairment) of the presence of the hyperlipidemia could be excluded by standard laboratory tests.

On the basis of the density of the LDL subfraction profiles and plasma lipid concentrations, 35 subjects from 13 families were selected to participate in this study. Twelve subjects were characterized by a buoyant LDL subfraction profile and 23 subjects by a dense LDL subfraction profile. The method of LDL subfractionation is described elsewhere in this article. All subjects with a buoyant LDL subfraction profile had normal lipids. Of the subjects with a dense LDL subfraction profile, 7 subjects were normolipidemic and 16 subjects were hyperlipidemic. This resulted in three groups of subjects; basal characteristics are summarized in Table 1. None of the subjects were on drug treatment or on a special diet, and none of the subjects used vitamin supplements.

Plasma Measurements
Fasting blood samples were collected into evacuated tubes containing K2-EDTA (1 mg/mL). The tubes were immediately placed on ice in the dark. Thirty nonlocal participants were visited at their homes. At 2 hours after blood sampling, plasma was separated from blood cells by centrifugation at 3600 rpm for 8 minutes at 4°C. Prior to the measurement of alpha-TOH and QH2-10 concentrations in plasma, saccharose as cryopreservative (final concentration 6 mg/mL) and BHT as antioxidant (final concentration 250 mg/mL) were added.

VLDL and LDL (d>1.019 g/mL) were isolated by ultracentrifugation. After removal of VLDL and LDL, cholesterol and triglyceride levels were measured in the infranatant and in total plasma. LDL was isolated from whole plasma by the polyethylene glycol 6000 method.30 Cholesterol and triglyceride levels were determined by enzymatic methods (No. 237574, Boehringer-Mannheim; No. 6669, Sera Pak, Miles, respectively). LDL cholesterol was calculated by subtraction.

Analysis of LDL Subfraction Profile
Each individual LDL subfraction profile was defined by a continuous variable, K, as described in detail by de Graaf et al.32 Briefly, LDL subfractions were separated by single-spin density-gradient ultracentrifugation, according to an earlier described method.33 After ultracentrifugation the LDL subfractions were visible as distinct bands in the middle of the tube. Up to five LDL subfractions could be distinguished. The tubes were photographed. Accurate documentation of the LDL subfraction distribution was obtained by scanning the slides on an LKB 2202 ultrascan laser densitometer (Pharmacia LKB).32 The relative peak heights of the LDL subfractions on the scans were used to calculate parameter K as a continuous variable, which best describes each individual LDL subfraction profile. A negative value (K<0) reflects a more dense subfraction profile and a positive K value (K>0) a more buoyant profile.32

Oxidation of LDL
Plasma isolation was immediately followed by LDL isolation by density-gradient ultracentrifugation (40,000 rpm for 18 hours at 4°C) using an SW40 rotor (Beckman).34 After isolation of total LDL, the protein content of LDL was measured by the method of Lowry et al.,35 with chloroform extraction to remove turbidity, using bovine serum albumin as a standard. The oxidation experiments were performed as described by Esterbauer et al.36 as modified by Princen et al.34 Briefly, the oxidation of LDL (60 ug apolipoprotein B100) was initiated by the addition of CuSO4 to a final concentration of 18 muM/L at 37°C. The kinetics of the oxidation of LDL was determined by monitoring the change of the 234-nm diene absorption in a thermostatically monitored UV spectrophotometer (Lambda 12, Perkin Elmer GmbH), equipped with a nine-position automatic sample changer. Each LDL preparation was oxidized twice in two separate oxidation runs on the same day. Every oxidation run was controlled by analyzing one reference LDL, prepared from a pooled plasma stored at -80°C. The interassay coefficients of variation for the oxidation parameters lag time and oxidation rate, and maximal amount of conjugated dienes formed per milligram of protein of the reference LDL amounted to 1.2%, 5.6%, and 4.7%, respectively (n=10). To
guarantee a high reproducibility of the oxidation assay, it appeared to be necessary to clean the quartz cuvettes thoroughly after every three oxidation runs. For this purpose, cuvettes were immersed in 2% (vol/vol) Hellmanex (No. 329.001, Hellma) for 30 minutes under continuous stirring on a hot plate at 80°C. Subsequently, cuvettes were thoroughly washed with deionized water for 15 minutes, followed by drying in a stream of filtered air.

**Determination of α-TOH, QH₂-10, and Q10 in Plasma and LDL**

To exclude any oxidation of QH₂-10, antioxidants were determined in material as fresh as possible. Consequently, plasma separation was immediately followed by LDL isolation by density-gradient ultracentrifugation (40,000 rpm for 18 hours at 4°C). LDL was isolated by cautious aspiration. Before extraction, BHT was added to the LDL preparations to a final concentration of 250 μg/mL. The concentrations of Q₁₀ (oxidized form of coenzyme Q₁₀) in plasma and LDL were determined by HPLC (Spectra Physics model 8800) with UV detection at 275 nm, sequentially followed by electrochemical detection (Decade, Antec) for the determination of α-TOH and QH₂-10.79 In each run, samples obtained of group 2 and/or group 3 subjects were blindly analyzed together with samples obtained of group 1 subjects. Deoxygenated and transition-metal-free aqueous solvents were used. All treatments were performed on ice, in the dark, and under nitrogen.

Immediately after isolation, plasma or LDL (200 μL) was mixed with 2.0 mL ice-cold methanol. Subsequently, 4.0 mL ice-cold n-hexane was added and the mixture was vortex-mixed for 2 minutes. To exclude artefact due to instability, samples were extracted in series of maximal 10. After centrifugation for 2 minutes at 3600 rpm at 4°C, the hexane upper layer was collected and the extraction procedure was repeated. Both hexane layers were pooled and dried under a flow of nitrogen within 45 minutes at room temperature. The residue was stored at −20°C until injection. For at least 4 hours in the dark at 4°C (99.5±3.1% to initial concentrations; n=6). QH₂-10 concentrations were stable during storage in the presence of 250 μg BHT/mL for up to 1 week at −80°C (98.0±3.0% to initial concentrations; n=3). To use similar LDL preparations for both the oxidation and antioxidation experiments, we isolated LDL by density-gradient ultracentrifugation in the absence of BHT. QH₂-10 concentrations in LDL were 104.1±4.6% (n=8) compared with those concentrations measured in LDL isolated in the presence of BHT. Prior to all extractions, we added 250 μg BHT/mL. QH₂-10 concentrations in LDL, however, were similar to those received at extraction without BHT (99.0±2.1% [n=3] versus BHT method). Until hexane extraction of the LDL preparations, LDL was stored in 2 mL of methanol at −20°C after mixing. Under these conditions, QH₂-10 was found to be stable for at least 24 hours (100.4±3.8% to initial concentrations; n=6). Consequently, Q10 concentrations during the described storage conditions were stable. After the lipid extraction, the combined hexane layers were evaporated under N₂(g) and residues were stored at −20°C until analysis within 4 hours. During this time, QH₂-10 concentrations were stable (98.0±2.0% of initial concentrations; n=6). However, storage of the lipid residues for 24 hours at −20°C resulted in a 43.5±8.7% loss of the QH₂-10 content. Consequently, the Q10 content was increased.

**Stability of QH₂-10**

Because of the suggested instability of QH₂-10 in plasma and especially in LDL,20,25 we performed stability experiments to validate our results. In whole blood, QH₂-10 was found to be stable for at least 4 hours in the dark at 4°C (99.6±3.1% to initial concentrations; n=6). QH₂-10 concentrations in plasma were stable during storage in the presence of 250 μg BHT/mL for up to 4 hours. To exclude any oxidation of QH₂-10, antioxidants were determined in material as fresh as possible. Consequently, plasma separation was immediately followed by LDL isolation by density-gradient ultracentrifugation (40,000 rpm for 18 hours at 4°C). LDL was isolated by cautious aspiration. Before extraction, BHT was added to the LDL preparations to a final concentration of 250 μg/mL. The concentrations of Q₁₀ (oxidized form of coenzyme Q₁₀) in plasma and LDL were determined by HPLC (Spectra Physics model 8800) with UV detection at 275 nm, sequentially followed by electrochemical detection (Decade, Antec) for the determination of α-TOH and QH₂-10.79 In each run, samples obtained of group 2 and/or group 3 subjects were blindly analyzed together with samples obtained of group 1 subjects. Deoxygenated and transition-metal-free aqueous solvents were used. All treatments were performed on ice, in the dark, and under nitrogen.

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**Determination of Fatty Acids in LDL**

The concentrations of polyunsaturated (C₁₈:₂; C₂₀:₄) and of monounsaturated (C₁₈:₁) fatty acids in LDL were determined essentially as described in detail by de Graaf et al.11

**Statistical Analysis**

Prior to statistical testing, plasma triglycerides and VLDL-TG were transformed logarithmically because of skewing of the distributions. Differences in smoking and personal history of coronary artery disease were calculated by a χ² test. A one-way ANOVA was used to analyze the differences in the studied parameters between the three groups, followed by additional Tukey's multiple comparison tests. All values are presented as mean±SD. Associations between variables were calculated with Pearson's correlation coefficients. All statistical analyses, including logistic regression analysis, were performed using SPSS/PC software (SPSS Inc).
TABLE 2. Oxidation Characteristics of LDL Isolated From Normolipidemic and FCH Subjects

<table>
<thead>
<tr>
<th>Group 1 (n=12)</th>
<th>Group 2 (n=7)</th>
<th>Group 3 (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag time, min</td>
<td>70.4±11.4</td>
<td>66.8±14.1</td>
</tr>
<tr>
<td>Oxidation rate, nmol dienes·mg protein⁻¹·min⁻¹</td>
<td>22.5±3.3</td>
<td>23.8±2.0</td>
</tr>
<tr>
<td>dienes (t=0), nmol/mg LDL protein</td>
<td>175±12</td>
<td>178±11</td>
</tr>
<tr>
<td>dienesmax, nmol/mg LDL protein</td>
<td>710±70</td>
<td>738±59</td>
</tr>
</tbody>
</table>

See Table 1 for definitions of groups.

*P<.05 vs group 1; †P<.05 vs group 2.

Results

Plasma Lipids, Lipoproteins, and K Values

Mean concentrations of plasma lipids and lipoproteins and the characterization of the LDL subfraction profiles of the respective groups are presented in Table 1. The personal history of coronary heart disease increased from 0% to 31% in groups 1 to 3, respectively. Compared with groups 1 and 2, group 3 subjects had, by selection, higher concentrations of total cholesterol, LDL-C, VLDL-C, total triglycerides, and VLDL-TG, lower concentrations of HDL-C, and more negative K-values (Table 1).

Oxidation Characteristics of LDL and LDL Antioxidant Contents

The mean lag time of LDL for oxidation was shorter in group 3 than in group 1 (Tukey, P<.05) (Table 2). In group 2, an intermediate mean lag time was measured which, probably due to the small group size, did not differ significantly from both other groups. When we considered groups 2 and 3 as one group, on the basis of characterization of the LDL subfraction profile, we observed that the mean lag time of LDL to oxidation was shorter than that in group 1 (Table 3). Exclusion of the smokers in the respective groups did not affect the presented results. The lag time of total LDL to oxidation correlated with the density of the LDL subfraction profile of the subjects, expressed as the continuous variable K (r=.35, P<.05). The maximal amount of dienes formed per milligram of LDL protein during oxidation of LDL isolated from FCH subjects was lower than the amount of dienes formed in LDL from group 1. Oxidation rates of LDL and basal amounts of dienes in LDL were similar in the three groups.

We examined whether the oxidation characteristics of LDL could be attributed to differences in the basal α-TOH and QHₐ-10 concentrations. Plasma concentrations of QHₐ-10 were related to plasma concentrations of total cholesterol (r=.41, P<.01) and α-TOH (r=.41, P<.01). The absolute and relative concentrations of α-TOH, QHₐ-10, and Q10 in plasma and LDL are given in Table 4, while the most important results are presented in the Figure. No differences were found between the three groups in the LDL α-TOH content, quantified relatively to cholesterol, apolipoprotein, or PUFA (Table 4). While the LDL QH₂-10 content, quantified relatively to apolipoprotein and PUFA, tended to be lower in group 2 than group 1 (Tukey, P=.07), the Q10 content was significantly increased (Table 4). The fatty acid composition of LDL was similar in all three groups (Table 4). Remarkably, for all subjects with a dense LDL subfraction profile, both the ratio of LDL QH₂-10 to α-TOH and the redox status of coenzyme Q10 in LDL (QH₂-10/Q₁₀ ratio) were lower than the ratios in LDL from subjects having a buoyant LDL subfraction profile (Figure). In line with this, the redox status of coenzyme Q10 correlated with the density of the LDL subfraction profile (r=.37, P<.05). In nonsmoking subjects the ratio of LDL QH₂-10 to α-TOH was 0.05±0.01, 0.03±0.01 (P<.05 versus group 1), and 0.04±0.01 for groups 1, 2, and 3, respectively. Furthermore, exclusion of the smokers in the three groups resulted in a redox status of coenzyme Q10 in LDL of 3.4±1.0, 1.5±1.5 (P<.05 versus group 1), and 2.1±0.8 (P<.005 versus group 1), respectively. Even when groups 2 and 3 are considered as one group, characterized by a dense LDL subfraction profile (n=23), both ratios were significantly lower than those in group 1 (Table 4). Regarding our stability experiments (see “Methods”), it is unlikely that the observed differences in the ratios were due to oxidation ex vivo. A second indication that the shift in the redox status of coenzyme Q10 was not an artifact appeared in the similarity of the redox ratios in total plasma versus the indicated LDL fraction in the various groups. In the combined groups, we observed that 53% of the variability in oxidation rate could be predicted by the basal amount of dienes in LDL together with the basal LDL QH₂-10 to LDL PUFA content ratio (P<.0001).

Antioxidant Contents

Table 3. Characteristics of Buoyant and Dense LDL Isolated From Normolipidemic and FCH Subjects

<table>
<thead>
<tr>
<th>Buoyant LDL* (n=12)</th>
<th>Dense LDL† (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K value</td>
<td>0.06±0.07</td>
</tr>
<tr>
<td>Lag time, min</td>
<td>70.4±11.4</td>
</tr>
<tr>
<td>nmol QH₂-10/g apolipoprotein</td>
<td>700±138</td>
</tr>
<tr>
<td>nmol Q₁₀/g apolipoprotein</td>
<td>191±99</td>
</tr>
<tr>
<td>μmol Q₁₀/mmol cholesterol</td>
<td>32.9±14.0</td>
</tr>
<tr>
<td>QH₂-10/Q₁₀</td>
<td>3.2±1.0</td>
</tr>
<tr>
<td>Q₁₀-10/α-TOH</td>
<td>0.05±0.01</td>
</tr>
</tbody>
</table>

*LDL isolated from group 1 subjects; †LDL isolated from group 2 and 3 subjects.

Discussion

Total LDL in FCH subjects (group 3) was less well protected against copper-mediated oxidation than total LDL from normolipidemic relatives with a buoyant LDL subfraction profile (group 1). Even when the subjects of groups 2 and 3, all characterized by a dense LDL subfraction profile, were combined, their LDL was more prone to oxidation than the LDL of group 1 (P<.05). To our knowledge, this is the first time a reduced oxidation resistance of total LDL, instead of isolated LDL subfractions, is presented for subjects characterized by a dense LDL subfraction profile. Possibly, the conformation of dense LDL particles facilitates the accessibility of copper to the apolipoprotein B100, or the premature exhaustion of antioxidants, thereby explaining the increased susceptibility to oxidation.
Quantitatively, α-TOH is an important antioxidant in LDL. However, in line with observations of other groups with subjects on a normal diet, the susceptibility of LDL to oxidative modification was not related to the α-TOH content of LDL. From in vitro oxidation studies, QH$_2$-10 is shown to be an antioxidant of the first line. LDL that was isolated from normolipidemic subjects with dense LDL tended to have a lower QH$_2$-10 content, relative to the apolipoprotein B100 or PUFA contents, than LDL from subjects with a buoyant profile. In addition, the redox status of coenzyme Q10 (ratio of reduced to oxidized form of coenzyme Q10) was substantially reduced in dense LDL particles, independent of whether LDL was isolated from normolipidemic or hyperlipidemic subjects (Figure). The oxidation of QH$_2$-10 within LDL particles was reported to be accompanied by the formation of lipid hydroperoxides within LDL. Our data on the redox status of coenzyme Q10 and the consequences concerning the initial degree of lipid peroxidation concur with data of Alleva et al. For normolipidemic subjects these authors showed that hydroperoxide concentrations were increased in dense LDL compared with buoyant LDL particles. An increased lipid hydroperoxide content in dense LDL may also explain the observed increased susceptibility of dense LDL to copper-mediated oxidation in vitro. In healthy subjects, elevated concentrations of plasma lipid hydroperoxides were measured, in vivo, when the redox status of coenzyme Q10 was reduced. Thus, the redox status of coenzyme Q10 in dense LDL appears to be a sensitive marker for oxidative changes that take place in LDL in vivo. By careful analysis, we have shown that the reduced redox status of coenzyme Q10 in groups 2 and 3, versus that in group 1, cannot be explained by a higher artificial oxidation of QH$_2$-10 during the several analysis steps. In this respect it is interesting to note the 3- to 30-fold-lower QH$_2$-10 concentration in LDL of normolipidemic subjects versus that in group 1, cannot be explained by a higher α-TOH radicals (reviewed in Reference 23). In line with results of Stocker and coworkers, our results stress the promising role of QH$_2$-10.

Whether a decrease in the redox status of coenzyme Q10 in LDL, due to oxidative modification of LDL, may indirectly protect the PUFAs against lipid peroxidation by efficient reduction of α-TOH radicals (reviewed in Reference 23). In line with results of Stocker and coworkers, our results stress the promising role of QH$_2$-10.

### Table 4. Concentrations of α-Tocopherol and Ubiquinol-10 in Plasma and Lipoproteins

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=12)</th>
<th>Group 2 (n=7)</th>
<th>Group 3 (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-TOH, μmol/L</td>
<td>27.8 ± 10.3</td>
<td>27.5 ± 5.5</td>
<td>42.5 ± 9.0*</td>
</tr>
<tr>
<td>mmol α-TOH/mol cholesterol</td>
<td>5.3 ± 1.7</td>
<td>5.0 ± 1.1</td>
<td>6.5 ± 1.4</td>
</tr>
<tr>
<td>mmol QH$_2$-10/mol cholesterol</td>
<td>1035 ± 600</td>
<td>1026 ± 599</td>
<td>1159 ± 479</td>
</tr>
<tr>
<td>μmol α-TOH/mol cholesterol</td>
<td>193 ± 81</td>
<td>180 ± 95</td>
<td>177 ± 69</td>
</tr>
<tr>
<td>QH$_2$-10, μmol</td>
<td>550 ± 101</td>
<td>461 ± 161</td>
<td>593 ± 228*</td>
</tr>
<tr>
<td>mmol QH$_2$-10/mol cholesterol</td>
<td>69 ± 32</td>
<td>86 ± 95</td>
<td>91 ± 34*</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
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<tr>
<td>mmol α-TOH/mol cholesterol</td>
<td>1.9 ± 0.4</td>
<td>2.1 ± 0.6</td>
<td>2.3 ± 0.5</td>
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<tr>
<td>μmol α-TOH/g apolipoprotein</td>
<td>14.3 ± 3.1</td>
<td>14.5 ± 2.6</td>
<td>15.2 ± 3.3</td>
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<tr>
<td>mmol α-TOH/mol PUFA</td>
<td>7.0 ± 1.6</td>
<td>6.8 ± 2.1</td>
<td>7.5 ± 1.5</td>
</tr>
<tr>
<td>μmol QH$_2$-10/mol cholesterol</td>
<td>95.3 ± 18.0</td>
<td>69.6 ± 39.0</td>
<td>92.2 ± 32.0</td>
</tr>
<tr>
<td>mmol QH$_2$-10/mol cholesterol</td>
<td>700 ± 138</td>
<td>480 ± 252</td>
<td>621 ± 204</td>
</tr>
<tr>
<td>μmol QH$_2$-10/mol PUFA</td>
<td>342 ± 88</td>
<td>227 ± 88</td>
<td>308 ± 100</td>
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<tr>
<td>mmol QH$_2$-10/mol cholesterol</td>
<td>32.9 ± 14</td>
<td>62.7 ± 25*</td>
<td>47.7 ± 22</td>
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<tr>
<td>mmol QH$_2$-10/g apolipoprotein</td>
<td>191 ± 99</td>
<td>351 ± 136*</td>
<td>284 ± 147</td>
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<tr>
<td>PUFA/C18:1</td>
<td>2.9 ± 0.5</td>
<td>3.2 ± 0.4</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>C18:2/C18:1</td>
<td>2.6 ± 0.5</td>
<td>2.8 ± 0.4</td>
<td>2.6 ± 0.3</td>
</tr>
</tbody>
</table>

See Table 1 for definition of groups. *P<.05 vs group 1.
Atherosclerosis, Thrombosis, and Vascular Biology

Volume 17, No 1  January 1997


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