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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 Cu^{2+} -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 3 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 ($R^2 = .73$, $P = .0001$). 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. (*Arterioscler Thromb Vasc Biol.* 1997;17:127-133.)

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

Familial combined hyperlipidemia (FCH), first identified by Goldstein et al,¹ is a genetically and metabolically heterogeneous common lipid disorder, associated with an increased risk for cardiovascular disease.^{1,2} 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.^{3,4} Small, dense LDL was generally found to be prevalent in patients with coronary heart disease.⁵⁻¹⁰

Studies on LDL oxidation revealed that small, dense LDL particles are more prone to oxidative modification than buoyant LDL.^{11,12} Oxidation of LDL is believed to play an important role in early atherosclerosis.¹³ After being oxidatively modified in the intima, LDL is probably taken up by scavenger receptors of macrophages.¹⁴ As a

result of uncontrolled uptake of oxidized LDL, macrophages are converted into cholesterol-rich foam cells, a hallmark of early atherosclerotic lesions.¹⁵ 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.^{16,17} Epitopes of oxidized LDL also were detected in human fatty streaks.^{18,19}

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.²⁰ Epidemiological studies suggested that high (>100 mg/d) α -TOH intake contributes to reduced risk of atherosclerosis.^{21,22} Although α -TOH is believed to be the major antioxidant in LDL responsible for the protection against oxidation in vivo,²⁰ 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 mevalonate pathway, having coenzymatic activity in the enzyme system of mitochondria, where it functions as an essential electron carrier in the respiratory system.²⁴ This antioxidant also is present in foods such as soybeans, walnuts, almonds, oils, fruits, and spinach.²⁵ Despite low concentrations in LDL (0.5 to 0.8 mol/LDL particle), compared with LDL

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From the Department of Medicine, Division of General Internal Medicine, University Hospital Nijmegen, Nijmegen, the Netherlands.

*Both Y.B.R. and S.J.H.B. contributed equally to this study.

Correspondence to Y.B. de Rijke, PhD, Bosch Medicenter, Department of Clinical Chemistry, Nieuwstraat 34, 5211 NL, 's Hertogenbosch, the Netherlands.

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Selected Abbreviations and Acronyms

α -TOH	= α -tocopherol
BHT	= butylated hydroxytoluene
FCH	= familial combined hyperlipidemia (4)
HDL-C	= HDL cholesterol
HPLC	= high-performance liquid chromatography
LDL-C	= LDL cholesterol
PUFA	= polyunsaturated fatty acids; sum of C18:2 and C20:4
Q10	= ubiquinone-10
QH ₂ -10	= ubiquinol-10
VLDL-C	= VLDL cholesterol
VLDL-TG	= VLDL triglycerides

α -TOH 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 peroxy 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.J.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 variability in plasma concentrations of lipids and cholesterol. Therefore, FCH families offer a unique opportunity to investigate the oxidation characteristics of both dense LDL from subjects with or without hyperlipidemia and buoyant LDL from normolipidemic subjects, all present in these families.

Methods**Chemicals**

Q10, D,L- α -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 (eg, 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 K₃-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 α -TOH and QH₂-10 concentrations in plasma, saccharose as cryopreservative (final concentration 6 mg/mL) and BHT as antioxidant (final concentration 250 μ g/mL) were added.

VLDL and IDL ($d \leq 1.019$ g/mL) were isolated by ultracentrifugation. After removal of VLDL and IDL, cholesterol and triglyceride levels were measured in the infranant and in total plasma. HDL was isolated from whole plasma by the polyethylene glycol 6000 method.³¹ 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 ($K \geq 0$) 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 al³⁶ as modified by Princen et al.³⁴ Briefly, the oxidation of LDL (60 μ g apolipoprotein/mL) was initiated by the addition of CuSO₄ to a final concentration of 18 μ mol/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%, and 4.7%, respectively (n=10). To

TABLE 1. Characteristics of Subjects, Plasma Concentrations of Lipids and Lipoproteins, and the LDL Subfraction Profile (ie, K value)

	Group 1 (n=12)	Group 2 (n=7)	Group 3 (n=16)
Age, y	41.4±12.7	39.4±12.9	46.2±11.8
Body mass index, kg/m ²	23.5±2.2	24.8±2.7	25.8±2.8
Smokers, >10 cigarettes/d, %	16.7	14.3	18.8
Personal history of coronary heart disease, %*	0	14.3	31.2
Total cholesterol, mmol/L	5.16±0.96	5.59±0.64	6.67±1.34†
Total triglycerides, mmol/L	1.30±0.53	1.60±0.26	3.66±1.15†‡
VLDL-C, mmol/L	0.36±0.17	0.64±0.18	1.51±0.52†‡
VLDL-TG, mmol/L	0.76±0.41	1.01±0.22	2.86±1.05†‡
LDL-C, mmol/L	3.28±0.82	3.87±0.61	4.25±1.30
HDL-C, mmol/L	1.34±0.28	1.04±0.22	0.87±0.14†
K value	0.06±0.07	-0.30±0.20†	-0.64±0.16†‡

Group 1 indicates buoyant LDL subfraction profile, normolipidemic subjects; Group 2, dense LDL subfraction profile, normolipidemic subjects; and Group 3, dense LDL subfraction profile, FCH subjects.

* $P < .0001$ (χ^2 test); † $P \leq .005$ vs group 1; and ‡ $P \leq .005$ vs group 2.

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 μ g/mL. The concentrations of Q-10 (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 QH₂-10.³⁷ 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 μ L) 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 artifact 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 within 4 hours. Just before injection onto the HPLC column, the residue was dissolved in the mobile phase, consisting of 22.5% (vol/vol) methanol and 77.5% (vol/vol) ethanol/isopropanol (95:5) with 20 mmol/L lithiumperchlorate as electrolyte. Twenty microliters of sample was injected onto an Inertsil ODS-2 column (200×3.0 mm; 5- μ m particle size) equipped with a reverse-phase guard column (10×2 mm) (both from Chrompack). α -TOH and QH₂-10 were eluted isocratically with the mobile phase at a flow rate of 0.35 mL/min. The eluate was monitored with an electrochemical detector. A VT-03 flow cell with GC working electrode (Antec) was used. The applied potential was 600 mV (versus Ag/AgCl in saturated LiCl). The interassay coefficients of variation for the determination of plasma and LDL concentrations of α -TOH were 1.3% and 4.5% (n=6), respec-

tively; of QH₂-10, 5.9% and 4.4% (n=8), respectively; of Q10, 3.1% and 5.6% (n=8), respectively.

Stability of QH₂-10

Because of the suggested instability of QH₂-10 in plasma and especially in LDL,^{26,38} 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 μ g 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 antioxidant 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 μ g 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% of 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.

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 χ^2 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

	Group 1 (n=12)	Group 2 (n=7)	Group 3 (n=16)
Lag time, min	70.4±11.4	66.6±14.1	60.4±8.1*
Oxidation rate, nmol dienes·mg protein ⁻¹ ·min ⁻¹	22.5±2.3	23.6±2.0	21.4±2.5
dienes (t=0), nmol/mg LDL protein	175±12	178±11	167±12
dienes _{max} , nmol/mg LDL protein	710±70	738±59	646±61*†

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 QH₂-10 concentrations. Plasma concentrations of QH₂-10 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, QH₂-10,

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).

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.³⁹

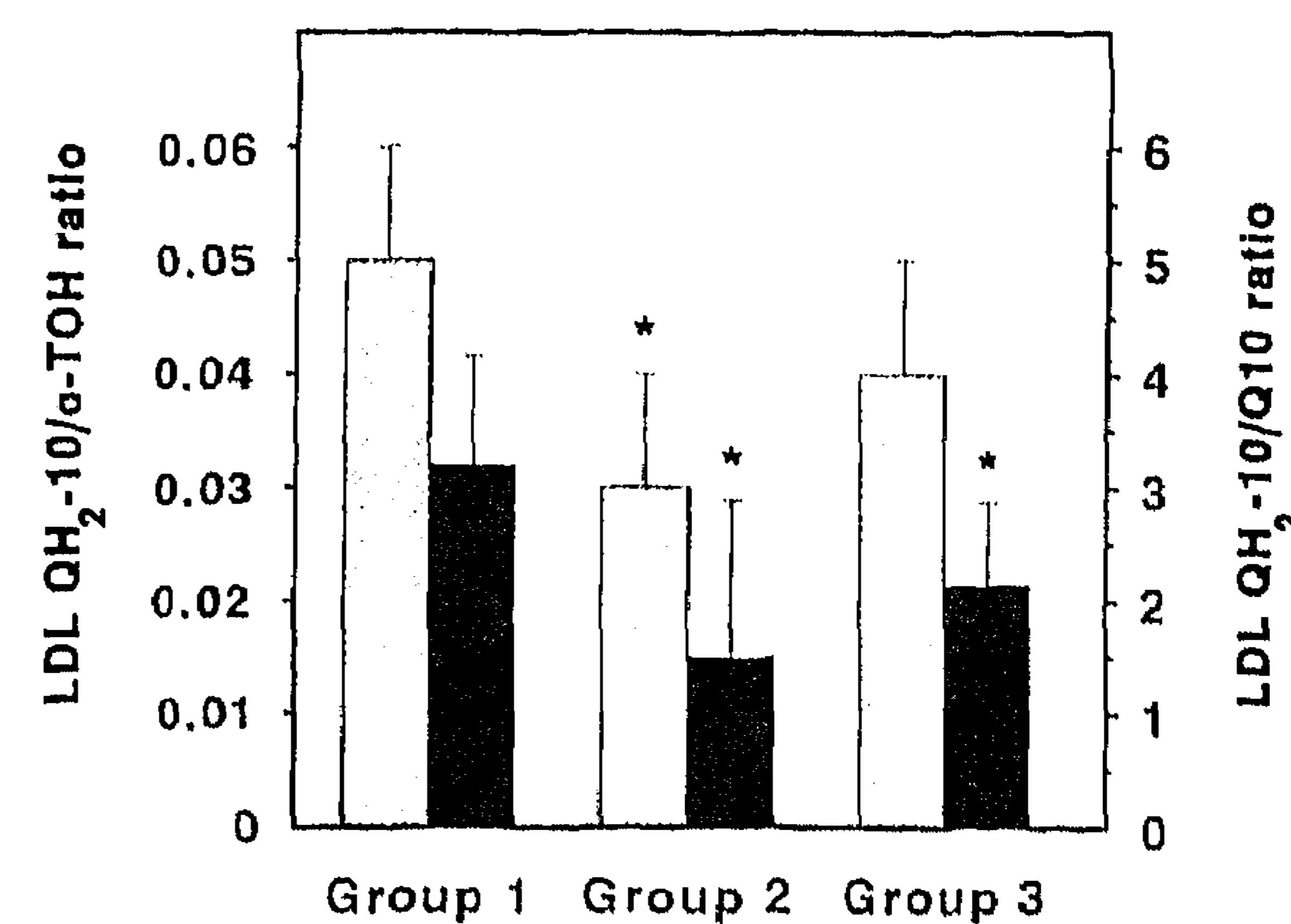
TABLE 3. Characteristics of Buoyant and Dense LDL Isolated From Normolipidemic and FCH Subjects

	Buoyant LDL* (n=12)	Dense LDL† (n=23)
K value	0.06±0.07	-0.54±0.24‡
Lag time, min	70.4±11.4	62.3±10.4§
nmol QH ₂ -10/g apolipoprotein	700±138	578±223§
nmol Q10/g apolipoprotein	191±99	304±144§
μmol Q10/mol cholesterol	32.9±14.0	52.3±24.0§
QH ₂ -10/Q10	3.2±1.0	1.9±1.0§
QH ₂ -10/ α -TOH	0.05±0.01	0.04±0.01‡

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

‡.0005<*P*<.005; §.01<*P*<.05.

Quantitatively, α -TOH is an important antioxidant in LDL.²⁰ 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.^{34,40-43} From in vitro oxidation studies, QH₂-10 is shown to be an antioxidant of the first line.²⁶ LDL that was isolated from normolipidemic subjects with dense LDL tended to have a lower QH₂-10 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 form 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 QH₂-10 within LDL particles was reported to be accompanied by the formation of lipid hydroperoxides within LDL.²⁶ 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.⁴⁴ 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.⁴⁵ In healthy subjects, elevated concentrations of plasma lipid hydroperoxides were measured, in vivo, when the redox status of coenzyme Q10 was reduced.⁴⁶ 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 QH₂-10 during the several analysis steps. In this respect it is interesting to note the 3- to 30-fold-lower QH₂-10 concentration in LDL of normolipidemic subjects³⁸ compared with concentrations measured in other studies^{26,27} and in the present study. We ascribe this to the lengthy isolation procedure of 3 days, including the dialysis step. To prevent loss or oxidation of LDL QH₂-



Relative QH₂-10 concentrations in LDL. LDL QH₂-10 content was quantified relative to the α -TOH content (left y axis, hatched bars), and the Q-10 content (redox status) (right y axis, filled bars). Group 1 indicates normolipidemic subjects with a buoyant LDL subfraction profile (n=12); Group 2, normolipidemic subjects with a dense profile (n=7); and Group 3, FCH subjects with a dense profile (n=16). **P*<.05 vs group 1.

10, LDL was not dialyzed in our study. This may explain why we did not find any relation between lag time and QH₂-10 content. In agreement with Kontush et al.,⁴⁷ we found that the basal LDL QH₂-10 to PUFA content ratio was negatively correlated with the rate of oxidative modification of LDL. QH₂-10 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,^{26,27} our results stress the promising role of QH₂-10.

Whether a decrease in the redox status of coenzyme Q10 in LDL, due to oxidation of QH₂-10, results in minimally oxidized LDL needs to be studied. Compared with native LDL, mildly oxidized LDL exerts important biological effects, at least in vitro. It is believed that these are involved in the early stages of atherosclerosis.⁴⁸ Minimally oxidized LDL was shown to stimulate the endothelial cell-mediated release of monocyte chemoattractant protein⁴⁹ and monocyte colony-stimulating factor.⁵⁰ On the other hand, it may be possible that the radicals that result in oxidation of coenzyme Q10 can also induce tryptophan residue destruc-

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

	Group 1 (n=12)	Group 2 (n=7)	Group 3 (n=16)
Plasma			
α -TOH, μ mol/L	27.6 \pm 10.3	27.5 \pm 5.5	42.5 \pm 9.0*
nmol α -TOH/mol cholesterol	5.3 \pm 1.7	5.0 \pm 1.1	6.5 \pm 1.4
QH ₂ -10, nmol/L	1035 \pm 600	1020 \pm 569	1159 \pm 479
μ mol QH ₂ -10/mol cholesterol	193 \pm 81	180 \pm 95	177 \pm 69
Q-10, nmol/L	350 \pm 101	461 \pm 161	593 \pm 228*
μ mol Q-10/mol cholesterol	69 \pm 32	85 \pm 35	91 \pm 34*
LDL			
nmol α -TOH/mol cholesterol	1.9 \pm 0.4	2.1 \pm 0.6	2.3 \pm 0.5
μ mol α -TOH/g apolipoprotein	14.3 \pm 3.1	14.5 \pm 2.6	15.2 \pm 3.3
nmol α -TOH/mol PUFA	7.0 \pm 1.6	6.8 \pm 2.1	7.5 \pm 1.5
μ mol QH ₂ -10/mol cholesterol	95.3 \pm 18.0	69.6 \pm 38.0	92.2 \pm 32.0
nmol QH ₂ -10/g apolipoprotein	700 \pm 138	480 \pm 252	621 \pm 204
μ mol QH ₂ -10/mol PUFA	342 \pm 88	227 \pm 88	308 \pm 100
μ mol Q-10/mol cholesterol	32.9 \pm 14	62.7 \pm 25*	47.7 \pm 22
nmol Q-10/g apolipoprotein	191 \pm 99	351 \pm 136*	284 \pm 147
PUFA/C18:1	2.9 \pm 0.5	3.2 \pm 0.4	3.0 \pm 0.4
C18:2/C18:1	2.6 \pm 0.5	2.8 \pm 0.4	2.6 \pm 0.3

See Table 1 for definition of groups.

**P*<.05 vs group 1.

tion.⁵¹ This process occurs in two phases; the earliest phase is independent of α -TOH and plays an initial role in LDL lipid peroxidation.⁵¹

In conclusion, dense LDL particles from subjects with FCH are less resistant to oxidation in vitro than buoyant LDL from normolipidemic relatives. Compared with subjects with an overall buoyant LDL subfraction profile, the redox status of coenzyme Q10 was reduced in subjects with an overall dense LDL subfraction profile, independent of the plasma concentration of cholesterol or triglycerides. This suggests that QH₂-10 is an important indicator of oxidative modification in vivo. Future investigations to assess oxidative stress in subjects at risk for coronary heart disease should include the redox status of coenzyme Q10.

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References

- Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease, II: genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J Clin Invest.* 1973;52:1544-1568.
- Brunzell JD, Albers JJ, Chait A, Grundy SM, Groszek E, McDonald GB. Plasma lipoproteins in familial combined hyperlipidemia and monogenic familial hypertriglyceridemia. *J Lipid Res.* 1983;24:147-155.
- Austin MA, Brunzell JD, Fitch WL, Krauss RM. Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. *Arteriosclerosis.* 1990;10:520-530.
- Bredie SJH, Kiemeny LA, de Haan AFJ, Demacker PNM, Stalenhoef AFH. Inherited susceptibility determines the distribution of dense low-density lipoprotein subfraction profiles in familial combined hyperlipidemia. *Am J Hum Genet.* 1996;58:812-822.
- Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass pattern and risk of myocardial infarction. *JAMA.* 1988;260:1917-1921.
- Swinkels DW, Demacker PNM, Hendriks JCM, Brenninkmeijer BJ, Stuyt PMJ. The relevance of a protein-enriched low density lipoprotein as a risk for coronary heart disease in relation to other known risk factors. *Atherosclerosis.* 1989;77:59-67.
- Griffin BA, Caslake MJ, Yip B, Tait GW, Packard CJ, Shepherd J. Rapid isolation of low density lipoprotein (LDL) subfractions from plasma by density gradient ultracentrifugation. *Atherosclerosis.* 1990;83:59-67.
- Krauss RM. Low-density lipoprotein subclasses and risk of coronary artery disease. *Curr Opin Lipidol.* 1991;2:248-252.
- Tornvall P, Karpe F, Carlson LA, Hamsten A. Relationship of low density lipoproteins to angiographically defined coronary artery disease in young survivors of myocardial infarction. *Atherosclerosis.* 1991;90:67-80.
- Campos H, Genest JJ Jr, Blijlevens E, McNamara JR, Jenner JL, Ordovas JM, Wilson PW, Schaefer EJ. Low density lipoprotein particle size and coronary artery disease. *Arterioscler Thromb.* 1992;12:187-195.
- de Graaf J, Hak-Lemmers HLM, Hectors MPC, Demacker PNM, Hendriks JCM, Stalenhoef AFH. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arterioscler Thromb.* 1991;11:298-306.
- Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis.* 1992;93:189-199.
- Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med.* 1989;320:915-924.
- Steinbrecher UP, Witztum JL, Parthasarathy S, Steinberg D. Decrease in reactive amino groups during oxidation or endothelial cell modification of LDL: correlation with changes in receptor-mediated catabolism. *Arteriosclerosis.* 1987;7:135-143.
- Schwartz CJ, Valente AJ, Sprague EA, Kelley JL, Nerem RM. The pathogenesis of atherosclerosis: an overview. *Clin Cardiol.* 1991;14:11-16.
- Salonen JT, Ylä-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, Nyyssonen K, Palinski W, Witztum JL. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet.* 1992;339:883-887.
- Maggi E, Chiesa R, Melissano G, Castellano R, Astore D, Grossi A, Finardi G, Bellomo G. LDL oxidation in patients with severe carotid atherosclerosis: a study of in vitro and in vivo oxidation markers. *Arterioscler Thromb.* 1994;14:1892-1899.
- Ylä-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, Steinberg D. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest.* 1989;84:1086-1095.
- Ylä-Herttuala S, Rosenfeld ME, Parthasarathy S, Sigal E, Särkioja T, Witztum JL, Steinberg D. Gene expression in macrophage-rich human atherosclerotic lesions: 15-lipoxygenase and acetyl low density lipoprotein receptor messenger RNA colocalize with oxidation specific lipid-protein adducts. *J Clin Invest.* 1991;87:1146-1152.
- Esterbauer H, Gebicki J, Puhl H, Jürgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic Biol Med.* 1992;13:341-390.
- Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med.* 1993;328:1444-1449.
- Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med.* 1993;328:1450-1456.
- Bowry VW, Stocker R. Tocopherol-mediated peroxidation: the prooxidant effect of vitamin E on the radical-initiated oxidation of human low-density lipoprotein. *J Am Chem Soc.* 1993;115:6029-6043.
- Appelkvist E-L, Kalen A, Dallner G. Biosynthesis and regulation of coenzyme Q. In: Folkers K, Littaru GP, Yamamura Y, eds. *Biomedical and Clinical Aspects of Coenzyme Q.* Amsterdam, the Netherlands: Elsevier Science Publishers BV; 1991;6:141-150.
- Kamei M, Fujita T, Kanbe T, Sasaki K, Oshiba K, Otani S, Matsui-Yuasa I, Morisawa S. The distribution and content of ubiquinone in foods. *Int J Vitam Nutr Res.* 1986;56:57-63.
- Stocker R, Bowry VW, Frei B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does α -tocopherol. *Proc Natl Acad Sci U S A.* 1991;88:1646-1650.
- Mohr D, Bowry VW, Stocker R. Dietary supplementation with coenzyme Q₁₀ results in increased levels of ubiquinol-10 within circulating lipoproteins and increased resistance of human low-density lipoprotein to the initiation of lipid peroxidation. *Biochim Biophys Acta.* 1992;1126:247-254.
- Kleinveld HA, Naber AHJ, Stalenhoef AFH, Demacker PNM. Oxidation resistance, oxidation rate, and extent of oxidation of human low-density lipoprotein depend on the ratio of oleic acid content to linoleic acid content: studies in vitamin E deficient subjects. *Free Radic Biol Med.* 1993;15:273-280.
- Frei B, Kim MC, Ames BN. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc Natl Acad Sci U S A.* 1990;87:4879-4883.
- Assmann G. *Lipid Metabolism Disorders and Cardiovascular Disease: Primary Prevention, Diagnosis, and Therapy Guidelines for General Practice.* Munich, Germany: MMV-Medizin-Verl; 1993.
- Demacker PNM, Hijmans AG, Vos-Janssen HE, van't Laar A, Jansen AP. A study of the use of polyethylene glycol in estimating cholesterol in high density lipoprotein. *Clin Chem.* 1980;26:1775-1779.
- de Graaf J, Swinkels DW, de Haan AFJ, Demacker PNM, Stalenhoef AFH. Both inherited susceptibility and environmental exposure determine the low-density lipoprotein-subfraction pattern distribution in healthy Dutch families. *Am J Hum Genet.* 1992;51:1295-1310.
- Swinkels DW, Hak-Lemmers HLM, Demacker PNM. Single spin density gradient ultracentrifugation method for the detection and isolation of light and heavy low density lipoprotein subfractions. *J Lipid Res.* 1987;28:1233-1239.
- Princen HMG, van Poppel G, Voegelzang C, Buytenhek R, Kok FJ. Supplementation with vitamin E but not β -carotene in vivo protects low density lipoprotein from lipid peroxidation in vitro: effect of cigarette smoking. *Arterioscler Thromb.* 1992;12:554-562.
- Lowry OH, Rosebrough NH, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193:265-275.
- Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vitro oxidation of human low density lipoprotein. *Free Radic Res Commun.* 1989;6:67-75.
- Lang JK, Gohil K, Packer L. Simultaneous determination of tocopherols, ubiquinols, and ubiquinones in blood, plasma, tissue homogenates, and subcellular fractions. *Anal Biochem.* 1986;157:106-116.
- Tribble DL, van den Berg JJ, Motchnik PA, Ames BN, Lewis DM, Chait A, Krauss RM. Oxidative susceptibility of low density lipoprotein

- protein subfractions is related to their ubiquinol-10 and alpha-tocopherol content. *Proc Natl Acad Sci U S A*. 1994;91:1183-1187.
39. Tribble DL, Thiel PM, van den Berg JJM, Krauss RM. Differing α -tocopherol oxidative lability and ascorbic acid sparing effects in buoyant and dense LDL. *Arterioscler Thromb Vasc Biol*. 1995;15:2025-2031.
 40. Jessup W, Rankin SM, De Whalley CV, Hoult JR, Scott J, Leake DS. Alpha-tocopherol consumption during low-density-lipoprotein oxidation. *Biochem J*. 1990;265:399-405.
 41. Babiy AV, Gebicki JM, Sullivan DR. Vitamin E content and low density lipoprotein oxidizability induced by free radicals. *Atherosclerosis*. 1990;81:175-182.
 42. Dieber-Rotheneder M, Puhl H, Waeg G, Striegl G, Esterbauer H. Effect of oral supplementation with D- α -tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation. *J Lipid Res*. 1991;32:1325-1332.
 43. Reaven PD, Khouw A, Beltz WF, Parthasarathy S, Witztum JL. Effect of dietary antioxidant combinations in humans: protection of LDL by vitamin E but not by β -carotene. *Arterioscler Thromb*. 1993;13:590-600.
 44. Alleva R, Tomasetti M, Battino M, Curatola G, Littarru GP, Folkers K. The roles of coenzyme Q10 and vitamin E on the peroxidation of human low density lipoprotein subfractions. *Proc Natl Acad Sci U S A*. 1995;92:9388-9391.
 45. Frei B, Gaziano JM. Content of antioxidants, preformed lipid hydroperoxides, and cholesterol as predictors of the susceptibility of human LDL to metal ion-dependent and -independent oxidation. *J Lipid Res*. 1993;34:2135-2145.
 46. Bowry VW, Stanley KK, Stocker R. High density lipoprotein is the major carrier of lipid hydroperoxides in human blood plasma from fasting donors. *Proc Natl Acad Sci U S A*. 1992;89:10316-10320.
 47. Kontush A, Hübner C, Finckh B, Kohlschütter A, Beisiegel U. Low density lipoprotein oxidizability by copper correlates to its initial ubiquinol-10 and polyunsaturated fatty acid content. *FEBS Lett*. 1994;341:69-73.
 48. Berliner JA, Territo MC, Sevanian A, Ramin S, Kim JA, Bamshad B, Esterson M, Fogelman AM. Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. *J Clin Invest*. 1990;85:1260-1266.
 49. Cushing SD, Berliner JA, Valente AJ, Navab M, Parhami F, Gerrity R, Schwarz CJ, Fogelman AM. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proc Natl Acad Sci U S A*. 1990;87:5134-5138.
 50. Rajavashisth TB, Andalibi A, Territo MC, Berliner JA, Navab M, Fogelman AM, Lusis AJ. Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. *Nature*. 1990;344:254-257.
 51. Giessauf A, Steiner E, Esterbauer H. Early destruction of tryptophan residues of apolipoprotein B is a vitamin E-independent process during copper-mediated oxidation of LDL. *Biochim Biophys Acta*. 1995;1256:221-232.