Ex vivo low-density lipoprotein oxidizability and in vivo lipid peroxidation in patients on CAPD

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Background. Chronic renal failure is associated with accelerated atherosclerosis and a high incidence of cardiovascular disease. Oxidative modification of low-density lipoprotein (LDL) is considered a key event in atherogenesis.

Methods. We studied the ex vivo oxidizability of LDL exposed to Cu^{2+} ions (lag time, rate of propagation, maximum conjugated diene formation) and its relationship with LDL density, fatty acids, and antioxidants, along with plasma malondialdehyde (MDA) and autoantibodies against Cu^{2+}-, MDA-, and hypochlorous acid-modified LDL and plasma antioxidants in 17 continuous ambulatory peritoneal dialysis (CAPD) patients and 21 healthy control subjects.

Results. LDL α- and γ-tocopherol and total polyunsaturated fatty acid (PUFA) concentrations were significantly higher in the CAPD patients. LDL density was shifted to small, dense LDL. LDL oxidizability was comparable to that of healthy subjects. Lag time correlated positively with LDL α-tocopherol and inversely with both total PUFA concentrations and density; the rate of oxidation and LDL density correlated positively with total PUFA and total fatty acid concentrations, respectively. Ratios of autoantibody titers against oxidized to native LDL did not differ between the two groups. While plasma α- and γ-tocopherol concentrations and tocopherol to cholesterol ratios were significantly higher, vitamin C concentrations were very low in the CAPD patients. MDA concentrations were 1.7 times higher than in healthy subjects.

Conclusions. (1) Ex vivo LDL oxidizability is normal in CAPD patients as a result of efficient protection by LDL-associated lipophilic antioxidants, although the LDL composition is altered toward high oxidizability; and (2) the plasma antioxidant screen is insufficient due to impaired vitamin C status.

Cardiovascular events are the most frequent causes of death in patients with end-stage renal disease. Continuous ambulatory peritoneal dialysis (CAPD) patients, in particular, show a highly atherogenic risk profile [1]. Peritoneal protein losses and glucose reabsorption contribute to high triglyceride, high low-density lipoprotein (LDL), high Lp(a), and low high-density lipoprotein (HDL) cholesterol concentrations [2].

Oxidative modification of LDL is thought to play a key role in atherogenesis [3]. Oxidation of LDL is a lipid peroxidation chain reaction that is driven by free radicals. The different components of LDL, that is, phospholipids, cholesteryl esters, triglycerides, apolipoprotein B, and antioxidants, participate in this process. Based on an average molecular weight of 2.5 million, each LDL particle contains more than 2000 molecules of fatty acids. Approximately half of them are polyunsaturated fatty acids (PUFAs; 86% linoleic, 12% arachidonic, and 2% docosahexaenoic acid), rendering the LDL particle susceptible to oxidation [4]. The major antioxidant in LDL is α-tocopherol, of which about six molecules are present in the LDL particle. LDL is defined as the population of lipoproteins that can be isolated from plasma by ultracentrifugation within a density gradient of 1.019 to 1.063 g/mL and consists of subfractions differing in size, molecular weight, density, and composition [5]. Small, dense LDL (subfractions LDL-4 and -5) dominates in hypertriglyceridemia and is characterized by higher triglyceride, PUFA, and protein content, while free cholesterol, cholesteryl ester, phospholipid, and vitamin E contents are lower than in LDL-1, -2, and -3 [6]. Individuals with a predominance of small, dense LDLs exhibit an increased risk of cardiovascular disease [7].

The oxidation of PUFAs in LDLs leads to formation of aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal, which are capable of modifying key lysine residues on apolipoprotein B, the protein moiety of LDL, thereby producing chemical adducts that are potent immunogens [4]. Modifications of LDL by hypochlorous acid (HOCl), a product of activated neutrophils, have been found in human atherosclerotic lesions [8]. There is some, but still not convincing, evidence that the transition metal copper would play a role in atherogenesis by oxidizing LDL in vivo [3]. A profound immuno-

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Table 1. Patient characteristics

	Patients N = 17	Normal range[a]
Creatinine *mg/dL*	8.32 ± 2.32	0.6–1.1
Urea *mg/dL*	110 ± 22.4	10–50
Urate *mg/dL*	6.42 ± 0.94	3.4–7.0
Albumin *mg/dL*	4.01 ± 0.52	3.5–5.0
Cholesterol *mg/dL*	239 ± 52	50–200
Triglycerides *mg/dL*	236 ± 216	70–150
C-reactive protein *mg/dL*	10.1 ± 7.64	0–5

Results are expressed as mean ± SD.

[a] Used as reference intervals in the Clinical Core Laboratory of the Department of Internal Medicine, Karl-Franzens University of Graz

logic response to different epitopes of modified LDL produces autoantibodies, the presence of which is considered an indicator of in vivo LDL modification [9, 10]. Such autoantibody titers proved to be an independent predictor of atherosclerosis progression [9]. Plasma MDA concentrations, even though not an indicator of oxidation of a specific lipid moiety in plasma but rather an end product of peroxidation of different PUFAs with three or more conjugated double bonds [11], have been used in numerous studies as a marker of in vivo lipid peroxidation [12, 13].

The purpose of this study was (*1*) to simultaneously determine in a nonselected group of CAPD patients the ex vivo LDL susceptibility to a defined oxidative stress and in vivo LDL and plasma lipid peroxidation, using autoantibody titers against oxidized LDL and plasma MDA concentrations, and (*2*) to analyze the relationships between these variables and others known to favor oxidation, such as small, dense LDL, high PUFA content, and impaired antioxidant status.

METHODS

Patients

Seventeen patients (12 males and 5 females, 23 to 82 years of age) with end-stage renal disease, regularly attending the CAPD outpatient clinic of the Division of Clinical Nephrology and Hemodialysis, Department of Internal Medicine, University of Graz, were enrolled in the study. Time on CAPD ranged from 0.2 to 5.2 years. For CAPD treatment, 1.5 to 4.25% dextrose dialysate solutions were used with four to five exchanges of 2 to 2.5 L exchanges per day. Nine patients had type I diabetes and received insulin therapy. None of the patients took lipid-lowering drugs nor did they use vitamin supplements. Subject characteristics are shown in Table 1. The study protocol was approved by the Ethics Committee Institutional Review Board of the General and University Hospital and Faculty of Medicine (Karl Franzens University of Graz), and informed consent was obtained from the patients.

Control subjects

Twenty-one healthy staff members (10 males and 11 females, 24 to 53 years of age) living in the same area volunteered as control subjects. All were on a regular diet, and none took medications or vitamin supplements.

Blood collection and processing

After an overnight fast, blood was collected either into ethylenediaminetetraacetic acid (EDTA)- or lithium heparin-containing plastic tubes (Sarstedt Monovette®) and centrifuged immediately at 4°C at 2000 × g for 10 minutes. Plasma was separated, divided into aliquots, and kept at −80°C until analysis. For plasma vitamin C determination, 0.5 mL of heparin plasma was mixed with 4.5 mL of 5% (wt:vol) metaphosphoric acid within a maximum of 20 minutes after blood had been drawn. A 60% sucrose solution to give a final concentration of 0.6% was added prior to storage at −80°C to those aliquots that were further processed for LDL isolation [14]. This procedure has been shown to protect against losses of antioxidants and changes in oxidizability of LDL prepared from stored plasma [15].

LDL isolation and determination of ex vivo LDL resistance to oxidation

Low-density lipoprotein was prepared using a single-step discontinuous gradient ultracentrifugation in a Beckman NVT 65 rotor at 60,000 r.p.m. at 10°C for two hours as described previously [16]. LDL was filtered through a 0.45 μm sterile filter (Corning, Inc., Corning, NY, USA) into an evacuated glass vial (TechneVial, Mallinckrodt-Diagnostica, Petten, Holland). LDL oxidation was carried out as described previously [16]. Briefly, LDL that had been made EDTA free was used at a concentration of 0.1 μmol/L in phosphate-buffered saline (PBS) equal to 0.25 mg LDL total mass/mL. The oxidation process was induced by adding an aqueous solution of $CuSO_4$ to give a final concentration of 1.67 μmol/L. Changes in conjugated diene (CD) absorbance were monitored at 37°C every three minutes for up to six hours. For characterizing LDL oxidizability [4], lag time (defined as the time during which rapid CD formation is inhibited by antioxidants), maximum rate of propagation ($\Delta A/\Delta t$, maximum increase in CD formation per unit of time during the propagation phase), time to reach maximum rate of propagation, maximum CD formation, and time to reach maximum CD formation were determined from the CD absorbance curve, either graphically (lag time) or by calculation using Microsoft Excel.

Determination of LDL density, fatty acid, and antioxidant content

Low-density lipoprotein density was determined using an Anton Paar DMA 48 density meter (A. Paar Ltd., Graz,

Austria), which allows mechanical oscillation resonance frequency determination [17]. In each of the individual LDL samples, α- and γ-tocopherol, α- and β-carotene, lycopene, canthaxanthin, cryptoxanthin, and zeaxanthin/lutein concentrations were measured by high-performance liquid chromatography (HPLC) [18] and expressed as mol antioxidant per mol LDL. The fatty acid profile of LDL was determined by gas chromatography, and the results were expressed as mol fatty acid per mol LDL [19].

Determination of plasma antioxidants, malondialdehyde, and autoantibodies against oxidized LDL

Plasma α- and γ-tocopherol, α-and β-carotene, lycopene, canthaxanthin, cryptoxanthin, and zeaxanthin/lutein concentrations were determined by HPLC according to Vuilleumier et al [20], as were plasma MDA concentrations according to Wong et al [21] with minor modifications as described [13]. Plasma vitamin C concentrations were determined by HPLC in the laboratory of the Vitamin Research Department of Hoffmann-La Roche (Basel, Switzerland), using the method of Schüep and Keck [22] with an adaptation for use in plasma.

Autoantibody titers against native and oxidized LDL

Autoantibody (IgG) titers were determined in serum using a solid-phase immunoassay system [23]. The antigens were either native LDL protected against oxidation by EDTA (1 mg/mL) and butylated hydroxytoluene (BHT) (1 mmol/L) and used immediately after isolation by ultracentrifugation, or LDL that had been modified by Cu^{2+} ions [24], MDA [25], or hypochlorous acid [8]. Briefly, microtiter plates were coated with 1 ng/mL protein in PBS/EDTA/BHT at pH 7.4 and incubated overnight at 4°C. The antigen was removed, and the plate was washed four times with Dulbecco's PBS + 21.2 g NaCl/L + 0.5% Tween 20 (washing buffer). The remaining binding sites were blocked with Dulbecco's PBS, pH 7.4, and 2% bovine serum albumin (BSA) at room temperature for one hour. Serum samples (10 μL) were diluted 1:20, and 200 μL were pipetted into wells coated with native LDL or Cu^{2+}-, MDA-, or HOCl-modified LDL. After incubation at 37°C for two hours, wells were decanted and washed four times with washing buffer. Then, 150 μL of the conjugate (antihuman IgG-horseradish peroxidase in 1:10,000 dilution in PBS/BHT/EDTA/1% BSA) was added to each well, incubated at room temperature for 60 minutes, decanted, and washed four times with washing buffer and one more time with 0.15 mol/L phosphate-citrate, pH 5.0. Into each well, 100 μL of the substrate solution (10 mL 0.15 mol/L phosphate-citrate, pH 5.0, 100 μL tetramethylbenzidine 1 mg/mL in ethanol and 10 μL 30% H_2O_2) were pipetted. After incubation at room temperature for 15 minutes,

the reaction was stopped by the addition of 2 mol/L sulfuric acid, and the absorbances were read at 450 nm (with 620 nm being the reference wavelength) in a microtiter plate reader (Anthos Labtec Instruments, Salzburg, Austria). Results were expressed as arbitrary units (ΔOD 450 nm − 620 nm) and as the ratio of autoantibody binding to oxidized/binding to native LDL, using the results of binding to native LDL as the blank [9]. Unspecific binding was usually negligible.

Statistical analysis

Comparisons between the CAPD patients and control subjects were made by the Student *t* test. Linear regression analysis was applied for studying relations between different variables. Sigma Stat version 2.03 (SPSS Scientific Software, Erkrath, Germany) was used for all statistical procedures. $P < 0.05$ was considered significant. Values are presented as mean ± SD.

RESULTS

Patient characteristics are shown in Table 1. Mean serum cholesterol, triglyceride, and C-reactive protein (CRP) concentrations were clearly above normal, while albumin concentrations were well within the normal range.

Ex vivo LDL resistance to oxidation and in vivo lipid peroxidation

Plasma MDA concentrations were significantly higher in the CAPD patients than in the control subjects ($P = 0.001$; Table 2). In contrast, all three indices of LDL resistance to oxidation, that is, lag time, maximum rate of propagation (and the time when it occurred), and maximum CD absorbance (and the time when it occurred), were comparable in the CAPD patients and control subjects (Table 2).

Antioxidant concentrations in plasma and in LDL

Plasma. The CAPD patients had significantly higher plasma α- ($P = 0.003$) and γ-tocopherol ($P < 0.001$), as well as higher β- ($P = 0.001$) and α-carotene ($P = 0.02$) concentrations than the control subjects (Table 3). There was no significant difference in the other carotenoids. Standardization for cholesterol of the fat-soluble plasma antioxidant concentrations did not alleviate the significant differences except for α-carotene. In contrast to the fat-soluble antioxidants, plasma vitamin C concentrations in the CAPD patients were only less than half the concentrations of the control subjects (Table 3). In four patients, they were below the detection limit.

LDL. The α- and γ-tocopherol and different carotenoid concentrations, expressed as mol antioxidant per mol LDL, are shown in Table 3. While both α- and γ-tocopherol concentrations were higher ($P < 0.001$ and

Table 2. Indices of in vivo lipid peroxidation and ex vivo low-density lipoprotein (LDL) oxidizability

	Patients N = 17	Control subjects N = 21	P value[a]
In vivo lipid peroxidation			
Plasma malondialdehyde *μmol/L*	1.09 ± 0.37[b]	0.65 ± 0.21	0.001
Ex vivo LDL oxidizability			
Lag time *min*	61.2 ± 9.6	62.6 ± 7.4	NS
Rate of oxidation *ΔA/min*	0.0237 ± 0.0039	0.0240 ± 0.0052	NS
Time *min*	82.5 ± 11.7	83.1 ± 7.89	NS
Maximum conjugated diene absorbance	0.928 ± 0.110	0.861 ± 0.152	NS
Time *min*	113.9 ± 14.8	111.0 ± 9.5	NS

[a] Student *t* tests
[b] Results are expressed as mean ± SD

Table 3. Antioxidant concentrations in plasma and low-density lipoprotein (LDL)

	Patients N = 17	Control subjects N = 21	P value[a]
Plasma *μmol/L*			
Vitamin C	30.6 ± 33.1[b]	69.5 ± 16.6	<0.001
α-Tocopherol	31.7 ± 13.3	20.8 ± 6.51	0.003
α-Tocopherol:cholesterol[c]	5.02 ± 1.38	3.18 ± 0.88	<0.001
γ-Tocopherol	2.21 ± 1.34	0.30 ± 0.23	<0.001
γ-Tocopherol:cholesterol	0.35 ± 0.19	0.04 ± 0.03	<0.001
β-Carotene	0.31 ± 0.25	0.11 ± 0.08	0.001
β-Carotene:cholesterol	0.05 ± 0.04	0.02 ± 0.02	0.003
α-Carotene	0.05 ± 0.04	0.03 ± 0.04	0.02
α-Carotene:cholesterol	0.007 ± 0.005	0.005 ± 0.007	NS
Lycopene	0.11 ± 0.12	0.06 ± 0.03	NS
Canthaxanthin	0.08 ± 0.07	0.16 ± 0.18	NS
Cryptoxanthin	0.16 ± 0.18	0.10 ± 0.06	NS
Zeaxanthin/Lutein	0.55 ± 0.39	0.33 ± 0.21	NS
LDL *mol/mol*			
α-Tocopherol	12.46 ± 3.81	8.89 ± 2.17	<0.001
γ-Tocopherol	0.85 ± 0.55	0.49 ± 0.22	0.008
β-Carotene	0.25 ± 0.26	0.14 ± 0.13	0.001
α-Carotene	0.05 ± 0.04	0.03 ± 0.04	NS
Lycopene	0.04 ± 0.05	0.10 ± 0.09	NS
Canthaxanthin	0.04 ± 0.05	0.05 ± 0.03	NS
Cryptoxanthin	0.07 ± 0.07	0.07 ± 0.04	NS
Zeaxanthin/Lutein	0.17 ± 0.13	0.14 ± 0.07	NS

[a] Student *t* tests
[b] Results are expressed as mean ± SD
[c] Cholesterol concentrations were 6.18 ± 1.35 mmol/L in the CAPD patients and 6.54 ± 1.32 mmol/L in the control subjects

$P = 0.008$, respectively) in the CAPD patients than in the control subjects, a significant difference was not found for the carotenoids. In the CAPD patients, 1 mol of α-tocopherol was present per 87 ± 31 mol of PUFAs as compared with 102 ± 28 mol in the control subjects.

Low-density lipoprotein fatty acid concentrations

As shown in Table 4, the total fatty acid concentrations (total saturated, total monounsaturated, and total PUFA concentrations) were significantly higher in the CAPD patients than in the control subjects. There was no difference in the ratio of oleic to linoleic acid between the CAPD patients and control subjects.

Effects of LDL antioxidants, PUFAs, and density on LDL resistance to oxidation

There was a significant positive relationship between lag time and the LDL α-tocopherol concentrations ($r = 0.56$, $P = 0.02$; Fig. 1). The intercept of the regression equation, $y = 43.8 + (1.40 × α$-tocopherol), indicates that 43.8 minutes of the mean lag time of 61.2 minutes were not explained by the LDL α-tocopherol content. The relationship between lag time and LDL γ-tocopherol concentrations, which accounted only for about one tenth of the α-tocopherol concentrations, was not significant ($r = 0.46$, $P = 0.07$, data not shown); the same held true for the carotenoid concentrations.

Figure 2 shows a significant inverse relationship between lag time and LDL total PUFA concentrations ($r = -0.54$, $P = 0.03$). Among the individual fatty acids, arachidonic acid made the strongest contribution to this relationship ($r = -0.72$, $P = 0.001$). There was also a significant positive relationship between the rate of propagation and LDL total PUFA concentrations ($r = 0.70$, $P = 0.002$). Linoleic acid was the individual fatty acid that made the strongest contribution to this relationship ($r = 0.74$, $P = 0.001$). There was no relationship between maximum CD formation and LDL fatty acid concentrations. Also, no correlation between lag time and the ratio of LDL oleic to linoleic acid was observed (data not shown).

As presented in Figure 3, the LDL density of practically all patients was in the high range, indicating a predominance of small, dense LDL according to Dejager, Bruckert, and Chapman [5] (LDL-4, d = 1.039 − 1.050; LDL-5, d = 1.050 − 1.063). There was a significant inverse relationship between lag time and LDL density ($r = -0.59$, $P = 0.01$), and LDL density was positively related to the LDL total fatty acid concentrations ($r = 0.62$, $P = 0.007$; Fig. 3).

Autoantibody titers against oxidized LDL

Results of autoantibody binding to native LDL, Cu^{2+}-, MDA-, and HOCl-modified LDL are presented in Figure 4, along with the ratios of autoantibody binding to

Table 4. Fatty acid concentrations in low-density lipoprotein (LDL)

	Patients N = 17	Control subjects N = 21	P value[a]
Saturated			
C14:0 (myristic)	25.9 ± 10.3[b,c]	24.6 ± 5.39	NS
C16:0 (palmitic)	616 ± 97.8	522 ± 53.6	<0.001
C18:0 (stearic)	179 ± 25.2	155 ± 22.1	0.003
C20:0 (arachidic)	17.6 ± 2.48	22.8 ± 3.44	<0.001
C22:0 (behenic)	18 ± 2.5	23 ± 3.4	<0.001
Total saturated fatty acids	847 ± 124	732 ± 72	0.001
Monounsaturated (MUFAs)			
C16:1n-9 (palmitoleic)	50.1 ± 22.9	35.5 ± 10.6	NS
C18:1n-9 (oleic)	422 ± 126	351 ± 69.1	0.03
C18:1n-7 (vaccenic)	36.9 ± 12.0	30.0 ± 11.8	NS
Total MUFAs	521 ± 141	416 ± 75.7	0.006
Polyunsaturated (PUFAs)			
C18:2n-6 (linoleic)	823 ± 150	704 ± 109	0.008
C18:3n-3 (α-linolenic)	14.9 ± 8.96	9.89 ± 1.53	0.005
C20:4n-6 (arachidonic)	159 ± 54.1	125 ± 31.5	0.02
C22:6n-3 (docosahexaenoic)	26.9 ± 10.82	21.2 ± 10.0	<0.001
Total PUFAs	1000 ± 191	860 ± 128	0.01
Ratio oleic acid to linoleic acid	0.514 ± 0.130	0.507 ± 0.108	NS
Total fatty acids	2368 ± 381	2001 ± 203	<0.001

[a] Student t tests
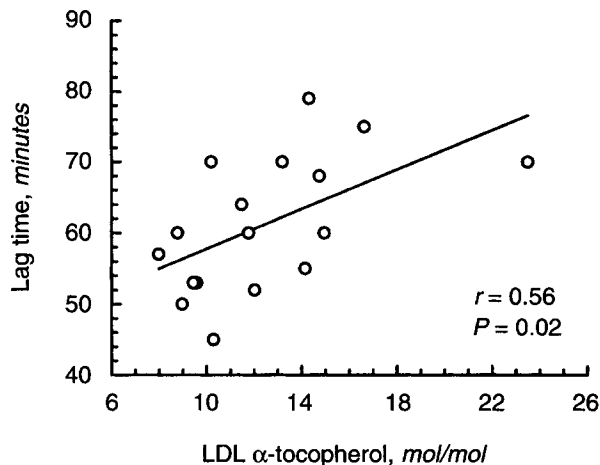[b] Results are expressed as mean ± SD
[c] Units are mol/mol

Fig. 1. Regression of lag time on low-density lipoprotein (LDL) α-tocopherol content in 17 continuous ambulatory peritoneal dialysis (CAPD) patients. The regression equation for α-tocopherol, $y = 43.8 + (1.40 \times$ α-tocopherol), indicates that 43.8 minutes of the mean lag time of 61.2 minutes are not explained by the LDL α-tocopherol content.

Cu^{2+}-, MDA-, and HOCl-modified/native LDL. CAPD patients showed a significantly lower autoantibody binding to Cu^{2+}- and MDA-modified LDL, but when corrected for differences in autoantibody binding to native LDL by using the above ratios, there were no significant differences between the two groups. Further analysis yielded significant correlations between the ratios of Cu^{2+}-oxidized/native LDL on one hand and MDA-modified/native LDL ($r = 0.57$, $P = 0.016$) and HOCl-modified/native LDL ($r = 0.61$, $P = 0.009$) on the other, and

between the ratios of HOCl-modified/native and MDA-modified/native LDL ($r = 0.84$, $P < 0.0001$).

DISCUSSION

This study demonstrates that LDL oxidizability is normal in CAPD patients as a result of excellent vitamin E and carotenoid status, even though (1) the PUFA content of LDL is higher than in healthy subjects, and (2) LDL density is shifted to small, dense LDL, both of which are known to increase LDL's susceptibility to oxidation. Autoantibody titers against oxidized LDL did not differ between the CAPD patients and control subjects. In concert, these data indicate that LDL is efficiently protected by lipophilic antioxidants. This is in agreement with data by Sutherland et al [26], who also found comparable lag times and 50% higher LDL vitamin E concentrations in CAPD patients.

In contrast to healthy subjects [4], yet in agreement with patients with malabsorption of fat in which a high proportion was vitamin E-deficient [27], CAPD patients showed a significant correlation between lag time and α-tocopherol content of LDL. However, a substantial proportion of lag time was not explained by α-tocopherol. Given the low concentrations in the LDL particle, it is not surprising that a significant correlation was not found for γ-tocopherol and the carotenoids. A protective action of β-carotene in vivo has only been demonstrated in β-carotene-deficient patients during repletion [13].

This study underscores that variables other than impaired antioxidant status are significant determinants of LDL susceptibility to oxidation. Kleinveld et al found
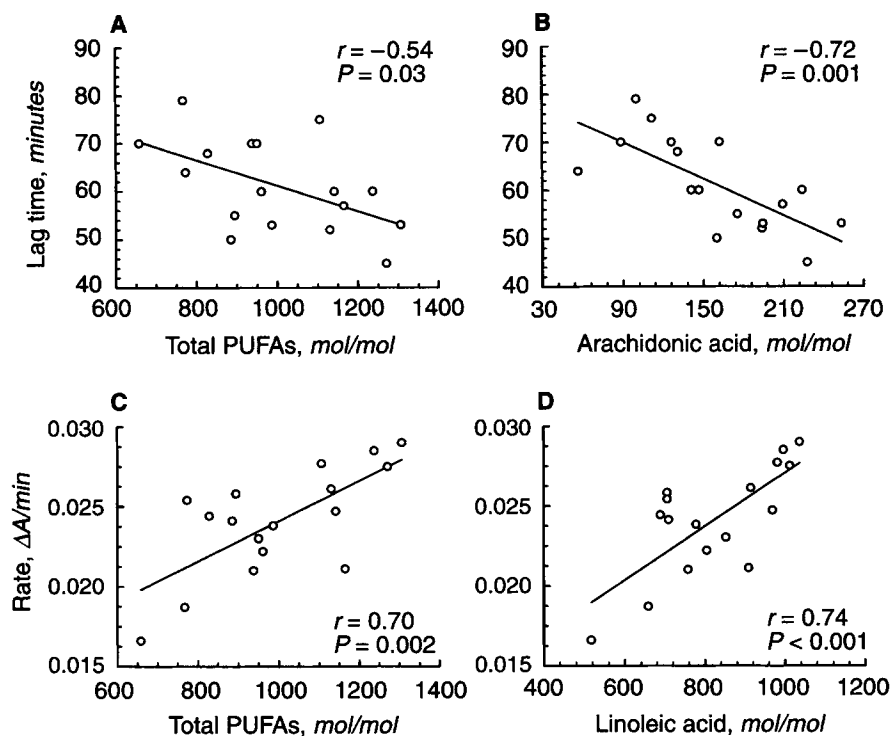
Fig. 2. Low-density lipoprotein (LDL) oxidizability and polyunsaturated fatty acid (PUFA) content. Shown are regressions of lag time on LDL total PUFA content (*A*) and arachidonic acid as the individual fatty acid with the strongest contribution to lag time (*B*) and regressions of the rate of propagation on LDL total PUFA content (*C*) and linoleic acid as the individual fatty acid with the strongest contribution to rate of oxidation (*D*). Results are presented for 17 CAPD patients.

LDL of patients with fat malabsorption to be rich in oleic and poor in linoleic acid and less easily oxidizable. From these data they concluded that the susceptibility of LDL to oxidation is determined by the ratio of oleic to linoleic acid [27]. Differences in the LDL PUFA content between CAPD patients and healthy subjects have been demonstrated for both oleic (higher in CAPD) and linoleic acid (lower in CAPD), resulting in ratios of oleic to linoleic acid of 0.77 in CAPD patients versus 0.49 in healthy subjects [27]. The CAPD patients of our study showed significantly higher LDL total fatty acid concentrations, but did not show a difference in the ratios of oleic to linoleic acid (0.51) compared to controls (0.51), providing another line of evidence that aids to explaining comparable LDL oxidizability.

In the CAPD patients, LDL density was shifted to the range of LDL-4 and -5, that is, the LDL subfractions summarized as small, dense LDL. In combined hyperlipidemia, the percentage of LDL-4 was positively correlated with plasma triglyceride and apolipoprotein B levels [5]. O'Neal et al demonstrated a 48% prevalence of small LDL in CAPD patients, as compared with 7% in control subjects, an inverse correlation between LDL particle size and plasma triglycerides, and a positive correlation between particle size and HDL cholesterol concentrations [28]. Our study provides new evidence by demonstrating a significant positive correlation between LDL density and total fatty acid concentrations. Among the individual fatty acids that showed a significant corre-

lation were palmitic acid, a saturated (*P* < 0.001) oleic acid, a monounsaturated (*P* = 0.04) and arachidonic acid, a PUFA (*P* = 0.015), which represented the three fatty acids that were present in LDL at the highest concentrations. In both normolipidemic and combined hyperlipidemic subjects, the LDL subspecies 3, 4, and 5 were moderately enriched in PUFAs, mainly linoleic and arachidonic acid [6]. It is concluded that the exceptionally rich vitamin E status in our CAPD patients efficiently counterbalanced the increased LDL PUFA content, resulting in normal LDL oxidizability.

Both lag time and rate of propagation correlated with LDL total PUFA concentrations; the correlation was negative for lag time and positive for rate of propagation. Arachidonic acid was the individual fatty acid that showed the closest correlation with lag time, whereas the rate of oxidation correlated best with linoleic acid. The stronger relationship between lag time, that is, the time before LDL enters the propagation phase of oxidation, and arachidonic rather than linoleic acid is in agreement with findings in healthy subjects [27]. It can be explained by the higher number of oxidizable double bonds in arachidonic (four) versus linoleic acid (two), thus rendering arachidonic acid more susceptible to oxidation. A PUFA disappearance kinetics study during autoxidation of LDL demonstrated that arachidonic acid is consumed about twice as rapidly as is linoleic acid [29], suggesting that at the time of maximum rate of propagation, arachidonic acid might be already partially
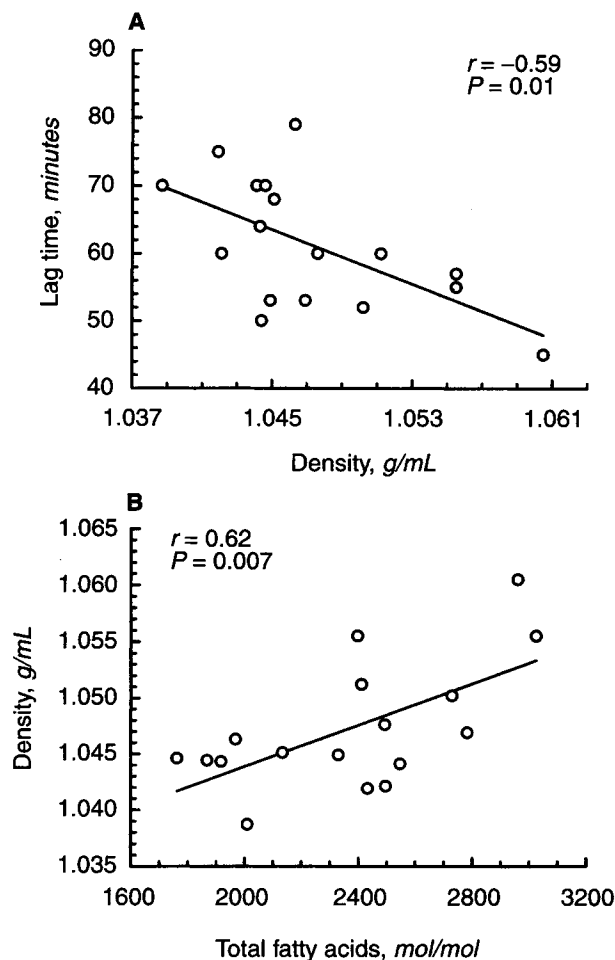
Fig. 3. Regression of lag time on LDL density (A) and regression of LDL density on LDL total fatty acid content (B). Results are presented for 17 CAPD patients. The normal range of LDL density is 1.019 to 1.063 g/mL; LDL subfractions, LDL-4 (d = 1.039 to 1.050) and LDL-5 (d = 1.050 to 1.063) are classified as small, dense LDL, according to Dejager, Bruckert, and Chapman [5].

consumed. It is postulated that PUFAs may affect the efficacy of α-tocopherol (lag time = $kx + a$; where k is the efficacy constant of α-tocopherol, x is the α-tocopherol concentration and a is the α-tocopherol-independent variable) [4], implicating that increasing amounts of PUFAs in the LDL particle will decrease the efficacy of one mol of α-tocopherol in protecting LDL from oxidation. LDL density, on the other hand, may affect the a of the equation, that is, the tocopherol-independent variable.

In contrast to normal ex vivo LDL susceptibility to oxidation, CAPD patients consistently exhibit high plasma MDA concentrations [30–33]. Among the methods available for assessing in vivo lipid peroxidation on a routine basis, MDA determination is regarded the most reliable one, provided it is determined by HPLC. However, MDA is not only formed nonenzymatically as an end

product of lipid peroxidation from n-3 and n-6 PUFAs with three or more conjugated double bonds, but also enzymatically from arachidonic acid via activation of the cyclooxygenase pathway [11]. Chronic activation of macrophages as an important source of reactive oxygen species (ROS) may play a significant role in CAPD [34]. Among other consequences of overproduction of ROS, nuclear factor-κB (NF-κB) will be activated and promote the gene expression of proinflammatory cytokines [35]. Recent data on hemodialysis patients suggest a relationship between MDA concentrations and interleukin-6 (IL-6) induction (abstract; Roob et al, *FASEB J* 14:A485, 2000). In any case, high circulating levels of MDA are considered undesirable, given its cytotoxic, immunogenic, and mutagenic properties [3, 9, 36].

Autoantibody formation against modified LDL was lower in the CAPD patients than in the control subjects, but there was no difference between the two groups when autoantibody binding to modified LDL was standardized for binding to native LDL. Close correlations were observed between the ratios of autoantibody binding to Cu^{2+}-, MDA-, and HOCl-modified/native LDL, suggesting that (*1*) LDL modifications that occur in vivo in CAPD patients are complex due to the simultaneous action of different products of oxidative stress, and that (*2*) a given patient responds to different types of modified LDL to a comparable extent.

In agreement with other studies [29, 37], plasma carotenoid concentrations were normal in the CAPD patients, and those of α- and γ-tocopherol were even higher than in the control subjects, indicating a sufficient lipophilic antioxidant screen against lipid peroxidation. However, recent data on chronic hemodialysis patients with comparable antioxidant status exposed to intravenous iron as a strong oxidant demonstrated that additional vitamin E exerts a further protective effect [38].

There are several potential sources of excessive reactive oxygen species production in CAPD that could chronically shift the oxidant-antioxidant balance in favor of the oxidants [34]. The net imbalance is aggravated by plasma vitamin C concentrations of less than half the normal values. This could easily explain increased in vivo lipid peroxidation frequently observed in CAPD patients. Vitamin C is a potent antioxidant that scavenges reactive oxygen species that might induce lipid peroxidation and efficiently regenerates the vitamin E radical formed during the action of vitamin E as a chain-breaking antioxidant, thus sparing vitamin E. In vitro studies demonstrated that vitamin C is superior to the other water-soluble antioxidants like bilirubin, uric acid, and protein thiols in protecting plasma and LDL against different types of oxidative stress [39]. In contrast to the LDL-associated antioxidants α-tocopherol, lycopene, and β-carotene, which lower the rate of lipid peroxidation but do not prevent its initiation, vitamin C neutral-
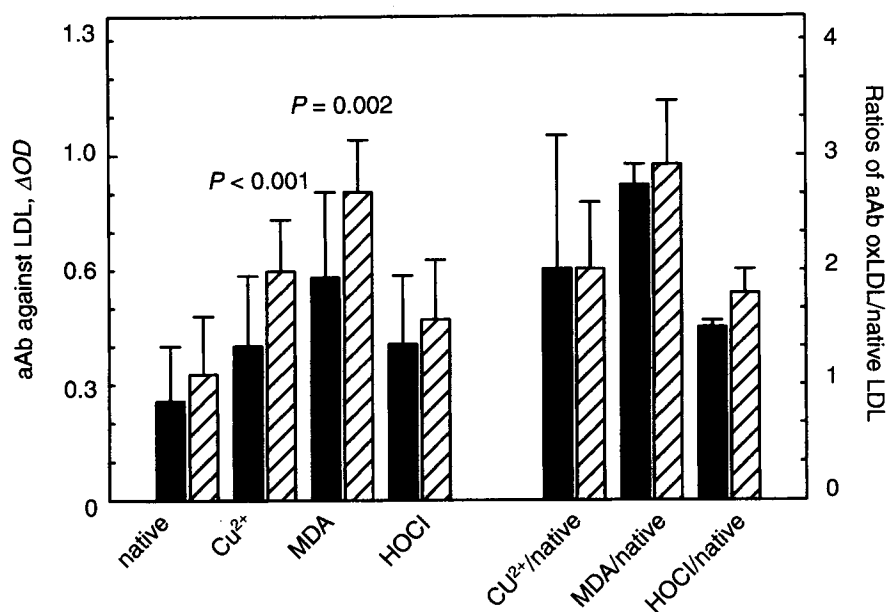
Fig. 4. Autoantibody (aAb) binding to native, Cu^{2+}-, MDA-, and HOCl-modified LDL in the CAPD patients (■; N = 17) and control subjects (▨; N = 21), along with the ratios of autoantibody binding to the three different types of modified LDL standardized for native LDL. Significant differences are indicated by P values. There were close correlations between the ratios of Cu^{2+}-, MDA-, and HOCl-oxidized/native LDL (discussed in the text).

izes oxidants in the aqueous phase before they can attack the lipids and lipoproteins. It is hence the only physiological antioxidant that can completely protect lipids in plasma and LDL against peroxidative damage [39]. Recently, vitamin C in combination with vitamin E has been shown to prevent IL-6 induction in response to oxidative stress (abstract; Roob et al, *FASEB J* 14:A435, 2000). Vitamin C status in CAPD patients is jeopardized by daily losses of approximately 40 to 60 mg into the dialysate, in which vitamin C is present at approximately 60% of plasma concentrations [37, 40].

In conclusion, as a result of highly efficient protection by LDL-associated lipophilic antioxidants, the isolated LDL particle of CAPD patients is no more susceptible to a defined ex vivo oxidative stress than is the LDL of healthy subjects, even though the LDL composition is shifted toward high oxidizability. The absence of differences in autoantibody titers against oxidatively modified LDL in this and another study [41] further suggests that in vivo oxidative modification of LDL with subsequent formation of autoantibodies does not occur more frequently in CAPD patients than in healthy subjects. However, severely impaired vitamin C status favors in vivo oxidative damage of plasma lipids, as suggested by the increased plasma MDA concentrations. Studies using additional methods for assessing in vivo lipid peroxidation more specifically, such as HPLC measurement of cholesteryl ester hydroperoxides, F_2-isoprostanes, and others, could help characterize the targets of lipid peroxidation in CAPD patients. These, in combination with intervention studies focusing on normalization of vitamin C status, are needed to prove that excessive in vivo lipid

peroxidation can be prevented long-term and, perhaps, reduce cardiovascular morbidity and mortality.

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