Resistance of Lipoproteins From Continuous Ambulatory Peritoneal Dialysis Patients to In Vitro Oxidation

Anatole P. Serdyuk and Richard E. Morton

Patients with end-stage renal failure on continuous ambulatory peritoneal dialysis (CAPD) develop abnormalities in plasma lipoproteins that may contribute to their increased risk for atherosclerosis. The oxidative modification of lipoproteins is considered to play a central role in atherogenesis. This study examines the susceptibility to oxidation in vitro of low- and high-density lipoprotein (LDL and HDL, respectively) obtained from long-term CAPD patients. CAPD LDL was less susceptible to copper-mediated protein derivatization (fluorescence) compared with control LDL. CAPD LDL and HDL displayed less copper-promoted conjugated-diene production and lipid peroxide generation, suggesting a greater resistance of CAPD lipoprotein lipids to oxidation. Autooxidation during long-term storage was also much lower in CAPD LDL and HDL. However, when 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP) was used to initiate oxidation, there was no difference in conjugated-diene generation between CAPD and the control. CAPD LDL contained slightly less oxidizable, polyunsaturated fatty acid, but the vitamin E content of CAPD and control LDL was equivalent. Our findings indicate that lipoproteins from uremic patients undergoing long-term CAPD are more resistant to in vitro oxidation than control lipoproteins.

UREMIC PATIENTS undergoing continuous ambulatory peritoneal dialysis (CAPD) have a high incidence of coronary heart disease and accelerated atherosclerosis.[1] Aberrations in plasma levels of lipids and lipoproteins that are often associated with an increased risk of coronary heart disease, such as increased plasma triglyceride and cholesterol, increased very–low-density lipoprotein and low-density lipoprotein (LDL) cholesterol and triglyceride, and decreased high-density lipoprotein (HDL) cholesterol, are common in CAPD patients.[2-4] Almost half of the patients on CAPD develop hypertriglyceridemia,[5] and about 30% of these develop hypercholesterolemia.[3] However, the mechanisms responsible for the abnormal lipid levels and high risk of coronary heart disease in CAPD have not been fully elucidated.

Based on in vitro data, LDL oxidation may play a causal role in atherogenesis.[6] Oxidized LDL loses the ability to be recognized by apolipoprotein B,E receptor and becomes a ligand for the scavenger receptors and other receptors on macrophages, and may facilitate cellular cholesterol accumulation.[6-8] The susceptibility of LDL to oxidative modification has been shown to correlate with the severity of atherosclerosis.[9,10] HDL, like LDL, is susceptible to oxidation,[11-13] leading to recognition by the scavenger receptor of macrophages and subsequent intracellular cholesterol accumulation.[11] The capacity of oxidized HDL to remove cholesterol from macrophages,[11] foam cells,[12] and fibroblasts[13] is significantly reduced compared with that of native HDL. The susceptibility of purified lipoproteins to oxidation in vitro is widely interpreted as an indicator of their atherogenic potential.[10-14]

The relationship between lipoprotein oxidation and the increased risk for coronary artery disease in uremic patients with and without dialysis remains unclear. Enhanced LDL susceptibility to in vitro oxidation and an increased level of lipid peroxidation are observed in uremic patients before dialysis, and hemodialysis intensifies the production of lipid peroxides.[15,16] However, the increased susceptibility to in vitro oxidation of LDL from hemodialysis patients has not been uniformly observed.[17] Serum and plasma antioxidant activities are lower in chronic renal failure patients with or without hemodialysis.[16,18,19] Consistent with these observations, antibodies against oxidatively modified LDL are present in the plasma of chronic renal failure patients with or without hemodialysis.[20] This suggests that oxidation of LDL in vivo is enhanced in these patients. The status of lipoprotein oxidation in CAPD-treated uremic subjects has been studied more limitedly. Maggi et al[15] reported that short-term (<10 months) CAPD treatment of uremic patients led to a marked reduction in LDL susceptibility to in vitro oxidation compared with that in uremic controls. However, despite the reduced oxidizability, they noted no decrease in the plasma content of oxidized LDL antigens that typify uremic subjects. The oxidative status of lipoproteins in patients on CAPD therapy for extended periods is less well characterized, but based on a limited number of subjects, it has been suggested to lead to a greater LDL oxidizability.[15] The aims of the present study were to investigate the susceptibility to in vitro oxidation of LDL and HDL isolated from patients on long-term CAPD treatment (>3 years) and to assess the oxidative status of these lipoproteins. CAPD subjects were segregated into normolipidemic and hypertriglyceridemic groups to further assess the impact of hyperlipidemia on lipoprotein oxidizability.

SUBJECTS AND METHODS

Study Populations

Plasma was obtained from residual clinical samples of patients undergoing CAPD treatment (n = 37). Samples were obtained randomly from a CAPD patient population that was 40% male and 60% female with a mean age of approximately 60 years. This population was 70% African-American and 30% Caucasian. All patients had been on CAPD treatment for at least 36 months and received 8 to 12 L Dianeal

From the Research Institute, Cleveland Clinic Foundation, Cleveland, OH.

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Address reprint requests to Richard E. Morton, PhD, Department of Cell Biology, Research Institute, NC 10, Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, OH 44195.

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dialysate per day (Baxter Travenol, Deerfield, IL). Patients typically received medication for hypertension (primarily calcium-channel blockers) and were treated with erythropoietin, phosphorus binders, and iron and vitamin D supplements. Control plasma (n = 19) was obtained from randomly selected donors to the Cleveland Clinic Foundation Blood Bank (45% male and 55% female) or from volunteers within our research facilities. Samples were not age-matched, since LDL oxidation status has been shown to be independent of subject age.[21]

CAPD plasma samples were segregated into normolipidemic (plasma triglyceride and cholesterol levels < 200 mg/dL, n = 23) and hypertriglyceridemic (triglyceride level between 200 and 500 mg/dL, n = 14) groups. The normal plasma triglyceride and cholesterol levels conformed to the National Cholesterol Education Program guidelines.[22] Mean triglyceride levels in normolipidemic CAPD (134.5 ± 8.7 mg/dL) and control (122.3 ± 10.4 mg/dL) groups and total cholesterol levels (173.6 ± 3.9 and 165.7 ± 5.5 mg/dL, respectively) did not differ. Lipoproteins from the hypertriglyceridemic CAPD group (mean plasma triglyceride, 297.9 ± 17.6 mg/dL) were used in selected experiments.

This study was approved by the Institutional Review Board, and informed consent was obtained when appropriate.

Plasma Collection

Blood samples were collected from patients and controls via venous puncture into sodium EDTA–containing tubes. All blood samples were immediately centrifuged at 2,000 × *g* for 15 minutes to obtain plasma. For some experiments, plasma samples were pooled before further processing.

Lipoprotein Isolation

Lipoproteins were isolated from plasma at 4°C by sequential ultracentrifugation[23] at solvent densities of 1.019, 1.063, and 1.21 g/mL to yield very–low-density lipoprotein, LDL, and HDL, respectively. All density solutions contained 0.02% EDTA. Lipoproteins were extensively dialyzed for 24 hours against a solution of 0.9% NaCl, 0.01% EDTA, and 0.02% NaN_3, pH 8.5, at 4°C in the dark. For oxidation studies, lipoproteins were dialyzed overnight against a solution of 0.9% NaCl, pH 7.4, at 4°C to remove EDTA, and were used for oxidation immediately. CAPD and control lipoprotein samples were obtained simultaneously and were oxidized on the same day.

Lipoprotein Oxidation

Lipoproteins (100 to 250 μg protein/mL) were oxidized immediately after dialysis with 2 to 5 μmol/L $CuSO_4$ (as noted in the text) or 200 μmol/L ABAP in 0.9% NaCl at 25°C, pH 7.4. Conjugated-diene production during copper-stimulated oxidation was determined by the increase in absorbance at 234 nm[24,25] using a continuously recording UV-visible spectrophotometer (Shimadzu UV 160U; Shimadzu Scientific Instruments, Colombia, MD) and quantified based on an extinction coefficient of 2.95×10^4 mol/L^{-1} · cm^{-1} at 234 nm.[25] The lag phase (t_{lag}, expressed in minutes), ie, the interval between the addition of $CuSO_4$ and the beginning of extensive oxidation, was measured on the basis of the intercept between the baseline and the tangent of the rapid oxidation phase.[26] Lipid peroxide generation was determined according to the method of El-Saadani et al[27]; the absorbance of lipoproteins determined immediately following the addition of color reagent was taken as the blank value for that sample. The concentration of lipid peroxides was calculated from the molar absorption coefficient of iodide determined at 365 nm (2.46×10^4 mol/L^{-1} · cm^{-1}).[27] Fluorescence intensity during LDL oxidation was measured at 430 nm with excitation at 360 nm (LS-3 fluorescence spectrometer; Perkin-Elmer, Norwalk, CT).[24,28]

Analytical Procedures

Protein content was determined by the method of Lowry et al[29] as modified by Peterson,[30] with bovine serum albumin as the standard. Total cholesterol was determined by a colorimetric, enzymatic method using the Cholesterol 100 reagent kit (Sigma Chemical). The free cholesterol level was measured by a colorimetric method with a standard enzymatic laboratory kit (Free Cholesterol C; Wako Pure Chemical Industries, Osaka, Japan). Cholesteryl ester was determined as the difference between total cholesterol and free cholesterol levels multiplied by 1.69 to correct for fatty acid content. Triglyceride content was measured by the glycerol phosphate oxidase-Trinder enzymatic method (Sigma Chemical). Vitamin A and E concentrations in plasma and LDL were analyzed by high-performance liquid chromatography on a C-18 reverse-phase column.[31]

LDL fatty acids were analyzed by gas-liquid chromatography after extraction, saponification, and transmethylation as previously reported.[32] Samples were fractionated on a 3% SP-2310/2% SP 2300 100/120 Chromosorb W/AW column (0.25 in × 6 ft; Supelco, Bellefonte, PA) with a temperature program from 185°C to 230°C at 2° increments per minute. An internal standard of behenic acid was added to all samples.

LDL electrophoretic mobility was determined by agarose gel electrophoresis[33] with transferrin as a mobility reference standard. Fragmentation of apolipoprotein B was assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 2.5% to 16% gradient gels (Isolab, Akron, OH).[34] The size of lipoprotein particles was determined by nondenaturing polyacrylamide gradient gel electrophoresis as previously described.[35]

Materials

Cupric sulfate was obtained from Fisher Scientific (Fair Lawn, NJ). 2,2′-Azobis(2-amidinopropane) dihydrochloride (ABAP) was from Wako Chemicals (Richmond, VA). All reagents for salt and buffer solutions and compounds for the cholesterol oxidase-iodide reagent for determination of lipid peroxides were purchased from Sigma Chemical (St Louis, MO). BCl_3-methanol for transmethylation of fatty acids was obtained from Supelco (Bellefonte, PA). Fatty acid standards were purchased from NuChek Prep (Elysian, MN).

Statistical Evaluation

Results are presented as the mean ± SEM. Differences between CAPD and control groups were evaluated statistically by Student's unpaired two-tailed *t* test. A *P* value less than .05 was considered significant.

RESULTS

Induced Lipoprotein Oxidation

During oxidative modification, polyunsaturated fatty acids with methylene-interrupted double bonds are converted to fatty acid hydroperoxides with conjugated double bonds (dienes) that absorb light at 234 nm.[16,24] The duration of the lag phase (t_{lag}) before the onset of aggressive oxidation reflects the capacity of lipoproteins to resist oxidation. Representative oxidation curves for CAPD and control LDL are shown in Fig 1. Analysis of multiple LDL preparations showed mean t_{lag} values of 110.7 ± 3.8 minutes (n = 4) and 80.7 ± 9.9 minutes (n = 5, $P < .05$) for normolipidemic CAPD and control LDL, respectively, showing a greater resistance of CAPD LDL to oxidative modification promoted by Cu^{2+}. The t_{lag} values for control LDL were comparable to those previously reported.[24] LDL isolated from normolipidemic and hypertriglyceridemic CAPD subjects

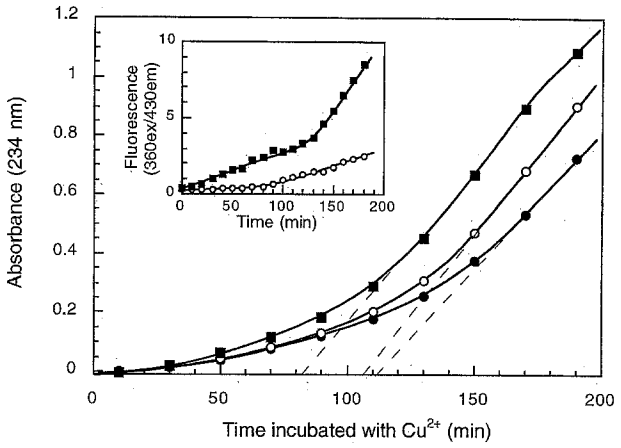

Fig 1. Conjugated-diene production in LDL incubated with copper. LDLs were dialyzed overnight against 2 changes of 0.9% NaCl solution without EDTA, pH 7.4. LDL 100 μg protein/mL was incubated with $CuSO_4$ 2 μmol/L at 25°C for the times indicated. Conjugated-diene generation was measured as the change in absorbance at 234 nm for normolipidemic (○) or hyperlipidemic (●) CAPD patients and control subjects (■). Absorbance at time zero was subtracted from all values. For clarity, only each tenth data point is shown; the solid line was fitted to all data points. The intercept of the baseline and the tangent of the rapid oxidation phase (----) is the t_{lag} value. These results are representative of 5 similar experiments that evaluated LDL from 7 normolipidemic and 6 hyperlipidemic CAPD patients and 11 control subjects. Inset: EDTA-free LDL (250 μg protein/mL) was incubated in the presence of $CuSO_4$ 5 μmol/L at 25°C. Fluorescence intensity of pooled normolipidemic CAPD (○, n = 3) or control (■, n = 3) LDL samples was measured every 10 minutes at 430 nm with excitation of 360 nm. Fluorescence at time zero was subtracted from all values. The solid lines are smooth curve fits of the experimental points.

had similar t_{lag} values (Fig 1). The slope of the propagation phase (rate of oxidation) was nearly the same for all three lipoprotein fractions (~3.4 nmol/mg LDL protein/min).

LDL fluorescence also increases considerably during LDL oxidation promoted by copper.[16,24,28] This generation of fluorophores primarily reflects the derivatization of apolipoprotein B amino groups.[28,36] Similar to that seen with lipid oxidation determinants, protein derivatization in the presence of copper was decreased for CAPD LDL compared with the control (Fig 1, inset), again showing the greater resistance of CAPD LDL to in vitro oxidation by metal ion–mediated pathways.

Similar to CAPD LDL, HDL from CAPD patients was more resistant to oxidation by copper (not shown). The t_{lag} (conjugated-diene production) was about 1.5-fold longer in both normolipidemic and hypertriglyceridemic CAPD HDL (70 to 75 minutes) compared with control HDL (50 minutes). The rate of oxidation in the propagation phase was similar for all HDLs.

Oxidation of LDL by copper requires the presence of preexisting lipid hydroperoxides for propagation and amplification to proceed.[37,38] ABAP, which facilitates autooxidation by generating hydroperoxyl radicals, does not require the presence of other initiating compounds.[14,37] In these studies, 200 μmol/L ABAP, instead of the higher concentrations more commonly used, was used to better assess the t_{lag} phase of oxidation. With ABAP as the oxidation initiator, there was no significant difference in t_{lag} between normolipidemic CAPD and control LDL (~55 minutes) or in the rate of oxidation (Fig 2). Similar results were noted for CAPD and control HDL (not shown). In contrast, hypertriglyceridemic CAPD LDL demonstrated a shorter t_{lag} (~20 minutes) than that of either normolipidemic group, but a nearly identical oxidation rate during the propagation phase (Fig 2). This suggests that LDL from hypertriglyceridemic CAPD subjects is more susceptible to oxidation in the presence of initiating compounds.

Generation of Lipid Peroxides in LDL Promoted by Copper

Lipid peroxides are the major initial reaction products of lipid peroxidation. Quantitation of peroxide formation assesses the oxidative status of polyunsaturated fatty acids in lipoproteins.[27] Lipid peroxide generation mediated by copper was significantly lower in LDL from both normolipidemic and hypertriglyceridemic CAPD patients compared with control LDL (Fig 3). The differences in lipid peroxide production were readily observed during the rapid oxidation phase between 0 and 18 hours, when the propagation rate was maximal. After 24 hours, peroxide generation in normolipidemic CAPD and control LDL was similar. The initial content, ie, before copper addition, of lipid peroxides in CAPD LDL and control LDL was near the lower limit of detection for the assay and was not measurably different between the two groups.

Suppression of LDL Oxidation by HDL

The protective effects of HDL on LDL oxidation in vitro are well established,[39,40] and may reflect a mechanism for minimizing LDL oxidation in vivo. Both CAPD HDL and control HDL were equally effective in delaying the onset of the rapid propagation phase of LDL oxidation mediated by copper (Fig 4).

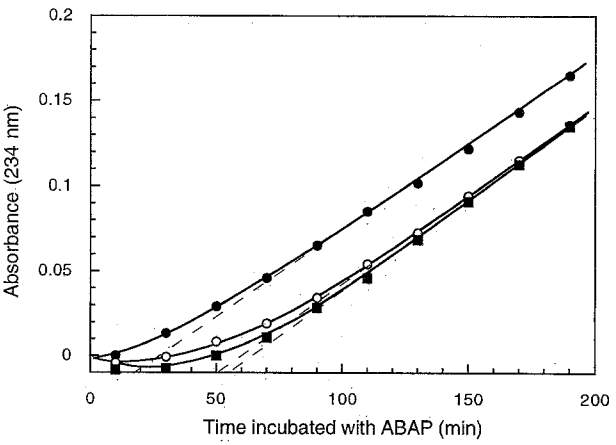

Fig 2. Conjugated-diene production in LDL incubated with ABAP. The LDL sample was dialyzed as indicated in Fig 1. LDL (100 μg protein/mL) was incubated with ABAP 200 μmol/L at 25°C for the indicated times. Absorbance at 234 nm in normolipidemic CAPD (○), hypertriglyceridemic CAPD (●), and control (■) LDL was monitored every 2 minutes. Absorbance at time zero was subtracted from all values. For clarity, only each tenth data point is shown. All time points were connected by the smooth curve fit shown. The intercept of the baseline and the tangent of the rapid oxidation phase (----) is the t_{lag} value. The data are representative of 3 experiments that analyzed LDL from 11 normolipidemic and 2 hypertriglyceridemic CAPD subjects and 6 controls.

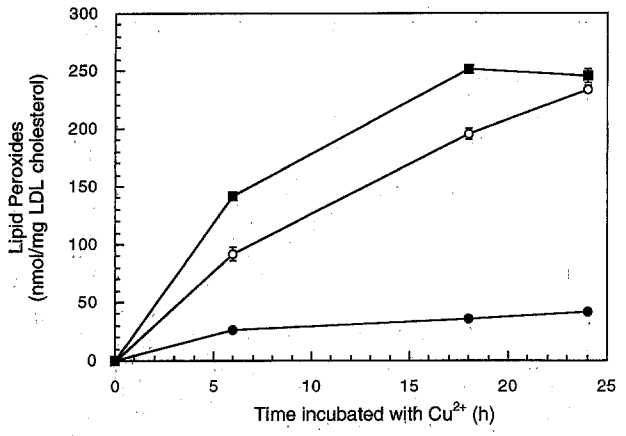

Fig 3. Lipid peroxide generation in LDL incubated with copper. EDTA-free LDL (1.5 mg cholesterol) was incubated in 0.9% NaCl solution containing $CuSO_4$ 5 μmol/L in a total volume of 1 mL for the indicated time. A 100-μL aliquot was taken at each time point for lipid peroxide determination. Lipoproteins became visibly aggregated at 18 and 24 hours. Blank values were determined at each time point to correct for sample turbidity. Pooled lipoproteins from 3 normolipidemic CAPD (○), 2 hypertriglyceridemic CAPD (●), and 3 control (■) subjects were used in the experiment. Data points shown are the mean of 2 determinations and are representative of 2 similar experiments. When not visible, error bars are contained within the symbols.

Lipoprotein Autooxidation During Storage

LDL, which was initially isolated and stored in EDTA-containing solutions, underwent marked autooxidation during extended storage at 4°C in saline without EDTA in the dark (Table 1). However, the generation of autooxidation products was different in CAPD and control LDL. Absorbance at 234 nm in CAPD LDL increased 127% during storage, whereas a 224% increase was noted for control LDL (Table 1). Fluorescence at 430 nm (360 nm excitation) in CAPD LDL increased threefold during storage, whereas it increased almost sixfold in control LDL (Table 1). These results show a greater resistance of CAPD LDL versus LDL from control subjects to autooxidation in the absence of metal ion mediators. These results mirror those already noted in the presence of copper.

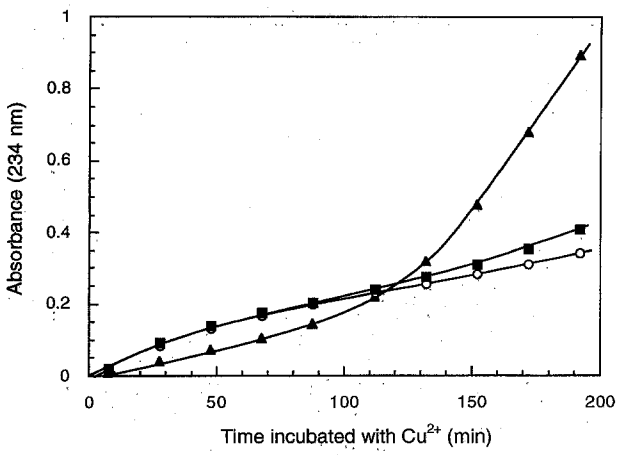

Fig 4. Effect of HDL on conjugated-diene production in LDL. EDTA-free LDL (100 μg protein/mL in 0.9% NaCl, pH 7.4) was incubated with $CuSO_4$ 2 μmol/L in a final volume of 1 mL for the indicated times at 25°C in the absence (▲) or presence of 100-μg protein/mL EDTA-free CAPD (○) or control (■) HDL. Formation of conjugated-diene was determined by measuring the increase in absorbance at 234 nm every 2 minutes. Conjugated-diene generation in LDL in the presence of HDL was determined after subtraction of the conjugated-diene generation in HDL alone. For clarity, only each tenth data point is shown; the line is fitted to all data points. These results are representative of 2 similar experiments.

Oxidation of HDL during storage was consistent with that seen for LDL. CAPD HDL underwent much less autooxidation than control HDL as determined by lipid or protein oxidation parameters (Table 1). HDL from both CAPD and control subjects was less susceptible to oxidation during storage than LDL.

LDL Chemical Characteristics

CAPD LDL from normolipidemic subjects differed from control LDL in several respects. The triglyceride content of CAPD LDL was elevated (10.1% of total lipid *v* 6.8% in controls), resulting in an increase of the triglyceride to cholesteryl ester ratio of the lipoprotein core from 0.14 in control to 0.24 in CAPD lipoproteins. Furthermore, perhaps in part due to this compositional change, CAPD LDL was larger (21% by volume) as determined by gradient gel electrophoresis. Fatty acid analysis of total LDL lipids showed that CAPD LDL contained more oleic acid and less linoleic acid than control lipoproteins (Fig 5). The content of all other fatty acids was not statistically different. This resulted in an increase of the saturated + monounsaturated to polyunsaturated ratio from 0.74 in control to 0.95 in CAPD LDL. Based on an analysis of the fatty acid content of individual lipid classes, this change in total fatty acid composition was primarily due to two factors. First, CAPD cholesteryl ester was enriched in oleate species. Second, although triglycerides were only slightly different in fatty acid content versus controls, CAPD LDL were enriched in triglycerides, which themselves contained proportionally more oleate than other lipid components. There was no statistically significant difference in the fatty acid composition of phospholipids in CAPD and control LDL (data not shown).

CAPD LDL was indistinguishable from control LDL on agarose gel electrophoresis (relative electrophoretic mobility = 0.83 compared with transferrin internal standard). Additionally, there was no evidence for apolipoprotein B fragmentation as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis of reduced samples (data not shown).

Plasma and LDL Antioxidant Vitamin Content

The concentration of vitamin A was significantly higher in the plasma of normolipidemic uremic patients undergoing CAPD (58% increase) versus control subjects (Table 2). However, LDL vitamin A levels were less than the detection limits. There was no significant difference between plasma and LDL vitamin E concentrations in CAPD and control groups even when corrected for the increased lipid content (greater molecular volume) of CAPD LDL (Table 2).

DISCUSSION

A major finding of this study is that lipoproteins obtained from uremic patients undergoing long-term CAPD therapy are more resistant to oxidation in vitro compared with control

Table 1. Generation of LDL and HDL Oxidation Products During Storage

Lipoprotein	Absorbance (234 nm) 0 d	Absorbance (234 nm) 40 d	Absorbance (234 nm) Change	Fluorescence (360 ex/430 em) 0 d	Fluorescence (360 ex/430 em) 40 d	Fluorescence (360 ex/430 em) Change
LDL						
CAPD	0.555 ± 0.018	1.261 ± 0.149*	0.706 (57.0%)	3.62 ± 0.62	11.02 ± 2.14*	7.40 (47.1%)
Control	0.554 ± 0.011	1.792 ± 0.152	1.238	3.30 ± 0.27	19.00 ± 2.46	15.70
HDL						
CAPD	0.425 ± 0.018	0.488 ± 0.008†	0.063 (25.4%)	0.98 ± 0.08	2.52 ± 0.14‡	1.54 (22.9%)
Control	0.443 ± 0.019	0.691 ± 0.043	0.248	1.20 ± 0.21	7.93 ± 1.11	6.73

NOTE. Freshly isolated LDL or HDL (100 μg protein/mL) was dialyzed against 0.9% NaCl, pH 7.4, to remove EDTA and separated into 2 aliquots. Absorbance at 234 nm and fluorescence intensity of 1 aliquot were determined immediately after sample dilution. The second aliquot was stored for 40 days at 4°C in the dark; afterward, absorbance and fluorescence were measured. The percent difference for the change in absorbance or fluorescence of CAPD LDL or HDL during storage relative to control lipoproteins is shown in parentheses. Data are the mean ± SEM of 5 CAPD and 4 control experiments. For these experiments, individual and pooled LDL or HDL samples were used that represented lipoproteins isolated from 12 CAPD and 10 control subjects.

*$P < .05$, †$P < .01$, ‡$P < .001$: CAPD *v* control groups.

lipoproteins when promoted by copper. CAPD LDL and HDL oxidation by copper was characterized by a significantly increased t_{lag}, but similar rates of oxidation in the propagation phase. The same kinetics were observed with both conjugated-diene and fluorescence end points. In contrast, the profiles of conjugated-diene generation were similar in normolipidemic CAPD and control LDL and HDL when oxidation was stimulated by the free radical generator ABAP. Hypertriglyceridemic CAPD LDL was more readily oxidized than control LDL by this reagent. The reduced capacity of copper to initiate oxidation in CAPD lipoproteins was mirrored by autooxidation levels in native lipoproteins stored for 40 days. Autooxidation during storage of LDL and HDL, measured as the conjugated-diene content and protein fluorescence, was lower in CAPD lipoproteins compared with the control. Overall, these data suggest a reduced susceptibility to oxidative stress in vitro of lipoproteins obtained from uremic patients on long-term CAPD.

Although the mechanisms of copper and ABAP action are not completely understood, some data suggest that initiation of copper-dependent oxidation requires the presence of trace amounts of lipid hydroperoxides.[37,38] However, Lynch and Frei[41] have questioned this conclusion. ABAP initiates oxidation with a hydroperoxyl radical that is similar in some respects to the chain-carrying radicals of polyunsaturated fatty acid oxidation, and does not require the presence of preformed lipid hydroperoxides.[14,37] The differences in the capacity of copper versus ABAP to oxidize CAPD lipoproteins suggest that CAPD lipoproteins may contain a lower concentration of initiating hydroperoxides. Yet we were unable to demonstrate a difference in the levels of these compounds in CAPD and control LDL. Alternatively, CAPD LDL may be less responsive to copper because of decreased copper binding to sites crucial for the initiation of oxidation[24] or because of enhanced binding (chelation) of copper by nonproductive sites, either of which would retard metal ion–mediated oxidation.

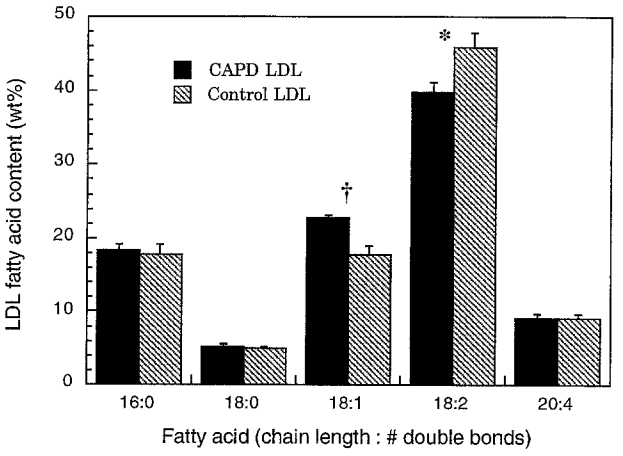

Fig 5. Fatty acid composition of LDL lipids determined by gas-liquid chromatography. The values (mean ± SEM) shown were determined from analysis of LDL isolated from 4 CAPD patients and 4 control subjects. *$P < .05$, †$P < .01$.

Oxidation of LDL produces fatty acid hydroperoxides from polyunsaturated fatty acids,[42] and LDL oxidation rates are linearly dependent on the concentration of polyunsaturated fatty acids in LDL.[14] Thus, LDL particles with a higher saturated + monounsaturated to polyunsaturated fatty acid ratio should be less susceptible to the oxidative stress. CAPD LDL was shown here to contain slightly lower levels of polyunsaturated fatty acids, which were accompanied by an increase in monoene species. The decreased percentage of polyunsaturated fatty acids (increased saturated + monounsaturated to polyunsaturated ratio) in CAPD LDL compared with control LDL may contribute to the resistance of CAPD LDL to oxidation. Clearly, the resistance of CAPD LDL to oxidation is not due to a lack of oxidizable substrate.

The susceptibility of LDL to oxidative modification also depends on the balance between polyunsaturated fatty acids and

Table 2. Vitamin A and E Content of Plasma and LDL

Group	Plasma Vitamin A (μg/dL)	Plasma Vitamin E (mg/dL)	LDL Vitamin E (μg/mg protein)	LDL Vitamin E (mol/mol LDL)
CAPD	99.8 ± 7.6*	1.05 ± 0.21	4.13 ± 0.43	4.90 ± 0.51
Control	63.0 ± 5.4	1.04 ± 0.10	4.36 ± 0.27	5.18 ± 0.32

NOTE. Vitamin A and E concentrations in normolipidemic CAPD patients and controls were determined by high-performance liquid chromatography. Data are the mean ± SEM of results from 6 CAPD and 8 control subjects.

*$P < .001$, CAPD *v* control.

antioxidants.[43] In this study, there was no difference in plasma or LDL vitamin E in CAPD and control groups. Interestingly, the plasma concentration of vitamin A was 1.6-fold higher in uremic patients on CAPD compared with control subjects. The role that this antioxidant plays in the lower oxidizability of CAPD lipoproteins remains to be determined. It should be kept in mind that LDL dialysis may result in a significant loss of lipophilic antioxidants.[44] However, it seems reasonable, given their similar lipid composition, that the loss of these antioxidants from CAPD and control LDL during processing would be comparable. Although vitamin E is increased in LDL during short-term CAPD therapy,[15] our data are consistent with the observation that this vitamin E enrichment is lost with further dialysis treatment.[15] However, we did not find subnormal vitamin E levels in LDL from long-term CAPD patients, as has been previously suggested to occur.[15]

It has been recently shown that calcium channel–blocking drugs, which are commonly used in CAPD patients, can exert weak lipid antioxidant activity in membrane systems.[45] However, the concentration of these drugs required to elicit these antioxidant effects greatly exceeds the pharmacologic concentration of these compounds in plasma. The impact of these drugs on our observations seems further unlikely, since it has been shown that there is no difference in the t_{lag} or propagation rate of LDL oxidation in vitro between uremic patients on calcium channel–blocking drugs and those not on these medications.[15,17]

In conclusion, the results of the present study show that LDL and HDL obtained from end-stage renal disease patients undergoing long-term CAPD therapy are more resistant to oxidative modification by copper than control lipoproteins. This was observed for lipoproteins from both normolipidemic and hypertriglyceridemic subjects. Thus, the previously recognized reduction in LDL oxidizability by short-term CAPD treatment[15] persists with continuing CAPD therapy. It remains to be determined why our results differ from those of Maggi et al,[15] who concluded that prolonged CAPD treatment increased the susceptibility of LDL to oxidation. The decreased LDL susceptibility to oxidation observed herein was not due to a higher content of vitamin E, nor does it appear to be the consequence of altered lipid composition, which is modified only to a minor extent. Thus, the risk for development of atherosclerosis and coronary heart disease in CAPD patients does not appear to be related to a greater susceptibility of lipoproteins to in vitro oxidation. A resistance to in vitro oxidation by metal ions has also been noted for LDL isolated from other patient populations with or at risk for the development of atherosclerosis.[17,26,46] These observations are in contrast to numerous studies showing that LDL oxidation often correlates with the risk for atherosclerotic disease, suggesting a causative role for oxidation in this process.[6,8-10,24] The resistance of LDL isolated from CAPD subjects to in vitro oxidation does not imply that lipoprotein oxidation is not important in the etiology of vascular disease in these patients, but rather that this putative role is not supported by in vitro measures of lipoprotein oxidizability.

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