Low-Density Lipoprotein (LDL) Oxidizability Before and After LDL Apheresis

Markus G. Donner, Klaus G. Parhofer, Werner O. Richter, and Peter Schwandt

Oxidation of low-density lipoprotein (LDL) plays a major role in the development of atherosclerosis. Hypercholesterolemia has been associated with enhanced in vitro oxidation of LDL, and lipid-lowering therapy reduces LDL oxidizability. In the present study, we investigated whether LDL apheresis performed with different techniques affects in vitro diene formation (lag phase) and modification of apolipoprotein B-100 (apoB). Baseline and posttreatment diene formation was correlated with the baseline pattern of plasma total fatty acids. We then performed a computer-simulation study to test the hypothesis that LDL apheresis–induced changes in LDL oxidizability are related to changes in the mass ratio between freshly produced and older LDL. In 19 patients aged 49 ± 7 years with heterozygous familial hypercholesterolemia (FH) regularly treated with either immunoadsorption, heparin-induced LDL precipitation (HELP), or dextran sulfate (DS) adsorption, we determined lipoprotein levels, the lag phase, apoB modification, and the fatty acid pattern in plasma samples drawn at the onset and termination of one LDL apheresis. LDL apheresis significantly decreased total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and triglycerides by 50.4%, 14.9%, 62.6%, and 33.6%, respectively. The lag phase increased by a significant mean of 9.8%; the charge of apoB was not altered. The lag phase before treatment positively correlated with the baseline concentration of plasma total palmitic, myristic, and oleic acid. The increase in the lag phase during treatment correlated with a high pretreatment concentration of lauric, linoleic, and docosahexanoic acid. The simulation study indicates that a temporary imbalance between two LDL compartments, one representing freshly secreted LDL and the other representing older LDL, could explain the observed increase in the lag phase after LDL apheresis. In conclusion, in patients with heterozygous FH, LDL apheresis performed with different techniques decreases the susceptibility of LDL to oxidation. This decrease may be related to a temporary mass imbalance between freshly produced and older LDL particles. Furthermore, the baseline fatty acid pattern influences pretreatment and posttreatment susceptibility to oxidation.

AN ELEVATED concentration of plasma low-density lipoprotein (LDL) is a major risk factor for atherosclerosis. Previously published studies indicate not only that an elevated LDL level is needed for the formation of foam cells in the arterial wall,[1] but that modification of LDL by acetylation or oxidation is a crucial step in the enhanced uptake of LDL by the scavenger receptor of macrophages.[2]

Enhanced oxidation of LDL due to the prolonged intravascular residence time has been established as one pathogenic mechanism for the development of premature coronary lesions in hypercholesterolemia.[3-5] Lipid-lowering agents not only decrease LDL cholesterol, but may also alter LDL susceptibility to oxidation.[6] Other factors that were shown to influence LDL oxidizability are the concentration of antioxidants, particularly vitamins C and E[7,8] and the pattern of fatty acids. While enrichment of LDL with saturated fatty acid (SFA) and monounsaturated fatty acid (MuFA) has been associated with decreased susceptibility to oxidation,[9,10] a high concentration of polyunsaturated fatty acid (PuFA) has been correlated with increased LDL oxidizability.[11] However, oxidation is not restricted to lipid peroxidation. During this process, apolipoprotein B-100 (apoB) can be modified also, leading to an increasing negative charge and fragmentation.[12]

In severe cases of LDL hypercholesterolemia, if combined drug therapy is not sufficient to reduce LDL cholesterol to an extent (ie, <2.58 mmol $\cdot$ L^{-1}) that prevents further progression of atherosclerotic lesions, LDL apheresis can be used as a potent additional treatment. LDL cholesterol can be reduced by a mean of 60%.[13-18] In combination with lipid-lowering agents, concentrations of 20% of the initial concentration may be reached.[19]

Several studies have addressed the question of whether LDL apheresis, in addition to decreasing LDL cholesterol, may also affect its susceptibility to oxidation.[20-22] The conclusions are limited by the small sample size and the fact that only one LDL apheresis system (dextran sulfate adsorption) was investigated.

The aim of our investigation was to study a larger sample of patients and to investigate the effect of different LDL apheresis techniques on LDL oxidizability. Further, the impact of the fatty acid pattern on posttreatment LDL oxidizability was investigated. We therefore determined pretreatment and posttreatment Cu-induced in vitro formation of conjugated dienes (lag phase) in 19 patients with heterozygous familial hypercholesterolemia (FH) treated with either immunoadsorption, heparin-induced LDL precipitation (HELP), or dextran sulfate (DS) adsorption. The fatty acid pattern was measured by high-performance liquid chromatography (HPLC). Fast protein liquid chromatography (FPLC) was used to detect changes in the charge of apoB during treatment. Finally, we performed a computer-simulation study to provide a hypothesis for the observed changes in oxidizability detected after LDL apheresis in this investigation and prior studies.

SUBJECTS AND METHODS

Patients

Nineteen patients (11 men and eight women) with heterozygous FH were included in the study. Despite the American Heart Association step I diet and simvastatin treatment at the maximally tolerable dose (except in six patients who did not tolerate hepatic hydroxymethyl glutaryl coenzyme A [HMG-CoA] reductase inhibitors), LDL cholesterol did not decrease sufficiently to prevent further progression of coronary

From Medical Department II, University of Munich, Klinikum Großhadern, Munich, Germany.

Submitted July 23, 1998; accepted January 6, 1999.

Current address: W.O.R., Institute for Lipoprotein Metabolism and Hemorrheology, Blumenstr. 6, 86949 Windach, Germany.

Address reprint requests to Markus G. Donner, MD, Medical Department II, University of Munich, Klinikum Großhadern, Marchioninistr. 15, 81377 Munich, Germany.

0026-0495/99/4807-0013$10.00/0

artery disease. Therefore, regular LDL apheresis was initiated at weekly intervals (except for five patients treated at biweekly intervals). All patients were currently nonsmokers. For the determination of LDL oxidation, apoB modification, and the fatty acid pattern in plasma, samples were drawn before apheresis and immediately after blood cells and plasma were returned to the patient. The investigations were performed under steady-state conditions (ie, regular LDL apheresis for at least 4 weeks).

LDL Apheresis

Patients were treated with immunoadsorption (n = 9), HELP (n = 4), or DS adsorption (n = 6). The procedures have been reviewed in detail elsewhere.[23] In brief, anticoagulation was performed with heparin (Braun, Melsungen, Germany; 2,500 to 5,000 IU as a bolus and 0 to 4,000 IU · h^{-1} continuously) and, in the case of plasma separation by centrifugation, additionally with citrate dextrose solution (2.5 to 3.0 mL · min^{-1}, ACD Formula B; Baxter Fenwal, Deerfield IL). For immunoadsorption, plasma was obtained by centrifugation (Cobe Spectra; Cobe, Lakewood, CO) or filtration (Mirosorb; Dialysetechnick, Ettlingen, Germany) and alternately pumped into two columns containing polyclonal anti–human apoB antibodies from sheep coupled to Sepharose 4B gel (LDL-Therasorb; Baxter Deutschland, Munich, Germany). In HELP, plasma was separated by a 0.55-μm polypropylene membrane filter (Haemoselect; Braun). The apoB-containing proteins were precipitated by addition of acetic acid buffer solution containing heparin 100 IU · mL^{-1} (pH 5.12) to the plasma and retained by a 0.4-μm polycarbonate membrane precipitation filter (Braun). The LDL-free plasma was passed through a polycellulose anion-exchange filter to remove the excess heparin in plasma (Braun) and then subjected to bicarbonate dialysis and ultrafiltration (Cuprophane filter; Braun). For DS adsorption, plasma obtained by filtration (Plasmaflux P2S; Fresenius, Oberursel, Germany) was alternately pumped into two columns (Liposorber LA-15; Kanegafuchi Chemical Industry, Osaka, Japan) each containing 150 mL cellulose-bound dextran sulfate, which adsorbs apoB-containing lipoproteins.

Lipoprotein Analysis and LDL Preparation

Total cholesterol and triglycerides were determined enzymatically in duplicate (Boehringer, Mannheim, Germany) with an automated analyzer (Epos; Eppendorf, Hamburg, Germany). High-density lipoprotein (HDL) cholesterol levels were measured enzymatically after precipitation of apoB-containing lipoproteins with sodium phosphotungstate and $MgCl_2$ (Boehringer). LDL for oxidation experiments was prepared by sequential ultracentrifugation (L5-75 ultracentrifuge equipped with Ti 50 rotor; Beckman, Fullerton, CA). Densities were adjusted with KBr. During the first step (density 1.019 g · mL^{-1}; 50,000 rpm for 24 hours), very–low-density lipoprotein and intermediate-density lipoprotein were separated and discarded. The infranatant was again subjected to ultracentrifugation (density 1.063 g · mL^{-1}; 50,000 rpm for 24 hours). A 1.5-mL volume of the supernatant containing LDL was dialyzed against 0.01 mol/L phosphate-buffered saline (PBS), pH 7.4, at 4°C in the dark for 24 hours.

LDL Oxidation

In vitro oxidation of LDL was quantified by monitoring the increase in diene absorption at 234 nm before LDL depletion of lipophilic antioxidants (lag phase). We used the method described by Esterbauer et al[24] with minor modifications. Following dialysis, the LDL stock solution was diluted with 0.01 mol/L PBS, pH 7.4, to produce a final concentration of 100 mg LDL cholesterol · L^{-1} PBS; 1 mL of this solution was mixed with 5 μL freshly prepared $CuCl_2$ solution (10 μmol · L^{-1}) to initiate lipid peroxidation. Diene formation was measured by recording the absorption at 234 nm every 10 minutes for 3 hours (Ultrospec plus; LKB, Cambridge, UK). Four samples and two controls (LDL solution without $CuCl_2$) were monitored at the same time. The lag phase was determined as the period before the rapid increase in diene formation (propagation phase).

FPLC of LDL

To detect changes in the protein moiety of LDL during LDL apheresis, aliquots of LDL prepared from samples drawn before and after treatment were subjected to FPLC as previously described by Vedie et al.[25] Briefly, separation was performed on a FPLC device equipped with two P-500 pumps (Pharmacia, Uppsala, Sweden) and an ion-exchange column (Pharmacia). A 20-μL volume of the LDL preparation was injected into the system and eluted with a linear gradient from buffer A (0.01 mol/L Tris hydrochloride, pH 7.4, with 1 mmol/L EDTA) to buffer B (buffer A plus 1 mol/L NaCl). Detection was made by an UV spectrophotometer at 280 nm, and the signal was recorded by a Pharmacia LKB REC 102 (Pharmacia). Retention time was used as a measure for the charge of the protein moiety.

Fatty Acid Pattern

Plasma total fatty acids were extracted into a chloroform/methanol solution, saponified, and derivatized as previously described by Shimomura et al.[26] For separation we used a HPLC device consisting of a gradient mixing pump (model 480; Gynkothek, Germering, Germany), a guard column (Beckman Ultrasphere, ODS, 4.6 mm × 4.5 cm), two reverse-phase columns (Beckman Ultrasphere, ODS, 4.6 mm × 25 cm), a column oven (temperature set to 60°C), a spectrofluorometer with a 20-μL flow cell (excitation 365 nm and emission 412 nm; Kontron, Neufahrn, Germany), and an integrator (Chromatopac RC 6 A; Shimadzu, Osaka, Japan). Quantification was made after two-point external standard calibration of the integrator. The concentrations were corrected for the internal standard heptadecanoic acid.

Simulation Study

Using a computer simulation, we tested the hypothesis of whether an altered ratio between freshly produced (less susceptible to oxidation) and older (more susceptible to oxidation) LDL particles could explain the differences in the lag phase. We assumed a hypothetical patient characterized by the following values: body weight, 70 kg; plasma LDL-apoB before LDL apheresis, 4 mmol · L^{-1}; plasma LDL-apoB after LDL apheresis, 1.33 mmol · L^{-1}; LDL-apoB production rate, 0.8 mmol · L^{-1} · d^{-1} (~0.32 mmol · kg^{-1} · d^{-1}); and LDL-apoB fractional catabolic rate, (FCR), 0.2 d^{-1}. These parameters are typical for a patient with heterozygous FH undergoing regular LDL apheresis therapy.[27,28] LDL-apoB metabolism was described by a two-compartment model (Fig 1) in which one compartment (compartment 1) represents freshly produced LDL (less susceptible to oxidation) and the other compartment (compartment 2) represents older LDL (more susceptible to oxidation). It was assumed that LDL is distributed equally between both compartments before LDL apheresis and that half of the freshly produced LDL in compartment 1 will leave the plasma before reaching compartment 2. We furthermore assumed that LDL apheresis decreases LDL-apoB in both compartments by 66% and does not alter the metabolic parameters characterizing the hypothetical patient. To evaluate to what extent the results of the simulation study depend on the assumed model, we repeated the simulation assuming that either 66% or 33% of total LDL-apoB is in compartment 1 and that the FCR of compartment 1 compared with compartment 2 is either elevated (0.6 d^{-1} *v* 0.2 d^{-1}) or decreased (0.3 d^{-1} *v* 0.4 d^{-1}). The simulations were performed with the SAAM II program (version 1.1; SAAM Institute, Seattle, WA).

Statistical Analysis

For statistical analysis, we used the SPSS/PC+ program (version 4.0 Microsoft; SPSS, Chicago, IL). Results are expressed as the mean ±

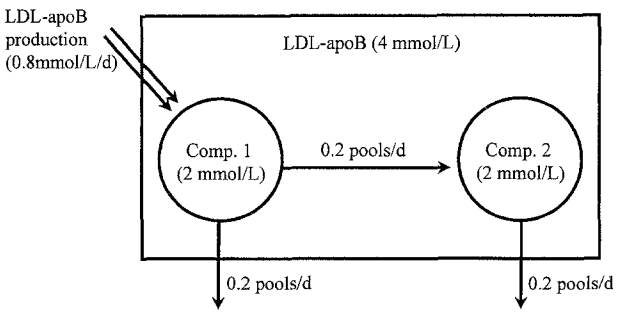

Fig 1. Model of LDL-apoB metabolism used in the simulation study. Comp., compartment.

SD. The *t* test for paired samples was applied to examine for statistically significant differences in parametric variables. For nonparametric variables, chi-square statistics were used.

RESULTS

Nineteen patients (11 men and eight women) with a mean age of 49 ± 7 years were studied. Lipoprotein concentrations and the lag phase before and after LDL apheresis are shown in Table 1. During the index apheresis, the mean plasma volume treated was 3,394 ± 969 mL. Total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides decreased significantly by 50.4%, 14.9%, 62.6%, and 33.6%, respectively. The lag phase increased by a significant mean of 9.8% (range, 0% to 24.2%; Table 1 and Fig 2). In the subgroup analysis, the lag phase increased from 62.6 ± 15.1 to 70.6 ± 19.9 minutes, 64.7 ± 9.4 to 67.0 ± 7.7 minutes, and 76.0 ± 43.4 to 81.0 ± 41.6 minutes in immunoadsorption (n = 9), HELP (n = 4), and DS adsorption (n = 6), respectively. Although the increase was found with all techniques used, the difference became statistically significant only in the immunoadsorption group ($P = .005$). The use of HMG-CoA reductase inhibitors did not affect the pretreatment ($P = .09$) or posttreatment ($P = .10$) lag phase or the change in the lag phase ($P = .71$). The retention time of apoB was 20 minutes in all patients and did not change during the procedure. The pattern of plasma total fatty acids before treatment is shown in Table 2. Calculated as the percent composition of SFA, MuFA, and PuFA, the distribution was 41.2% ± 2.2%, 29.1% ± 2.6%, and 29.5% ± 4.0%, respectively.

The lag phase before treatment positively correlated with the concentration of myristic acid ($r = .52$, $P = .02$), palmitic acid ($r = .48$, $P = .04$), and oleic acid ($r = .54$, $P = .02$). Pretreatment LDL cholesterol ($r = -.07$, $P = .69$) and the relative content of SFA ($r = .18$, $P = .45$), MuFA ($r = .39$, $P = .09$), and PuFA ($r = .34$, $P = .16$) were not correlated with the pretreatment lag phase. The increase in the lag phase during treatment positively correlated with the pretreatment concentration of lauric acid ($r = .58$, $P = .009$), linoleic acid ($r = .47$, $P = .04$), and docosahexanoic acid ($r = .56$, $P = .04$). In patients with a pretreatment lag phase above the median, we found a higher concentration of myristic acid (283.0 ± 122.6 *v* 151.9 ± 62.3 μmol/L, $P = .01$). Similarly, patients with a posttreatment lag phase above the median also had a higher concentration of myristic acid (247.2 ± 136.8 *v* 162.3 ± 67.0 μmol/L, $P = .01$). The decrease in LDL cholesterol ($r = .26$, $P = .62$) and the plasma volume treated ($r = -.02$, $P = .94$) did not correlate with an increased posttreatment lag phase.

Table 1. Lipoprotein Concentrations and Lag Phase Before and After LDL Apheresis (N = 19)

Parameter	Before Apheresis	After Apheresis	*P*
Cholesterol (mmol · L^{-1})	6.24 ± 1.01	3.10 ± 0.44	<.001
Triglycerides (mmol · L^{-1})	3.07 ± 1.01	2.04 ± 1.03	<.001
HDL cholesterol (mmol · L^{-1})	1.21 ± 0.21	1.03 ± 1.21	<.001
LDL cholesterol (mmol · L^{-1})	4.41 ± 0.95	1.65 ± 0.13	<.001
Lag phase (min)	64.8 ± 22.0	71.4 ± 23.2	.004

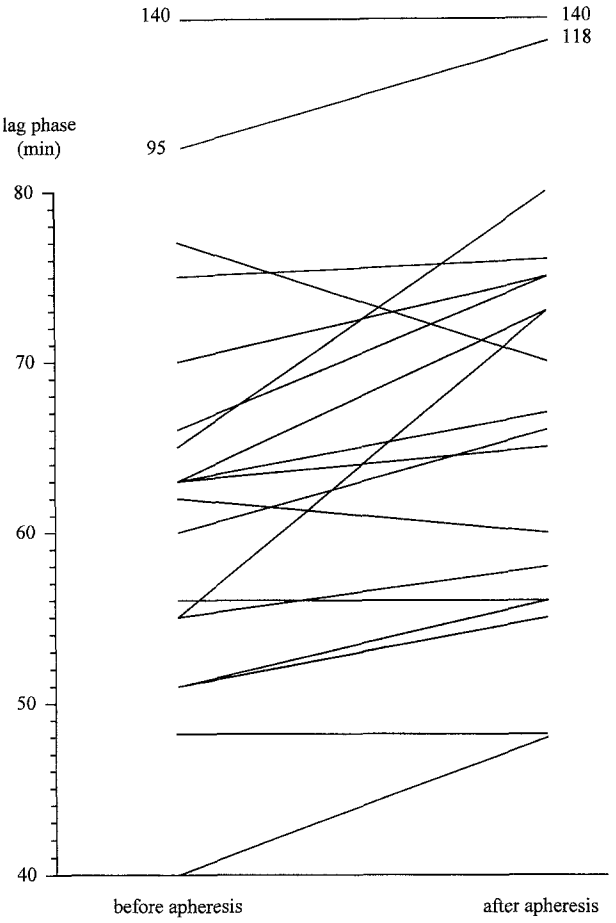

Fig 2. Lag phase (minutes) before and after treatment in 19 patients on LDL apheresis.

The results of the simulation study are shown in Fig 3, indicating the effect of LDL apheresis on the mass distribution between both compartments. The lines represent the rebound of total plasma LDL-apoB (compartments 1 and 2) and freshly produced (compartment 1) and older (compartment 2) LDL-apoB, respectively. Shortly after LDL apheresis, more LDL-apoB is in compartment 1 versus compartment 2, and thus in the compartment less susceptible to oxidation. When the initial assumption concerning the mass distribution between the two LDL compartments or the FCR were changed over a wide range of parameters, the principle finding of a delayed mass rebound in compartment 2 remained unchanged (data not shown). Only

Table 2. Concentration of Plasma Total Fatty Acids Before Apheresis and Correlation to the Lag Phase (N = 19)

Fatty Acid	Concentration (μmol/L)	% of Total	r_1	P	r_2	P
Lauric acid	80.9 ± 43.4	1.06 ± 0.57	.173	.48	.581	.01
Eicosapentanoic acid	144.8 ± 66.7	1.90 ± 0.87	.302	.21	.362	.13
Linolenic acid	85.3 ± 36.1	1.12 ± 0.47	.269	.27	.473	.04
Myristic acid	193.8 ± 100.4	2.54 ± 1.31	.519	.02	.348	.14
Docosahexanoic acid	55.0 ± 33.1	0.72 ± 0.43	.303	.29	.565	.04
Palmitoleic acid	327.3 ± 194.5	4.28 ± 2.55	.090	.09	−.043	.86
Linoleic acid	1,922.7 ± 50.86	25.12 ± 0.66	.260	.28	.153	.53
Palmitic acid	2,039.2 ± 316.7	26.69 ± 4.14	.480	.04	.110	.65
Oleic acid	1,917.6 ± 366.0	25.10 ± 4.79	.537	.02	.045	.86
Stearic acid	874.0 ± 208.7	11.44 ± 2.73	.082	.74	−.029	.90

NOTE. r_1 is the correlation coefficient between the pretreatment lag phase and fatty acid concentration; r_2 is the correlation coefficient between the increase in the lag phase during treatment and the fatty acid concentration.

when an extreme model is chosen (66% of apoB in compartment 2, with a low FCR), the rebound of apoB in compartment 2 may be faster than that of apoB in compartment 1. Furthermore, depending on the initial steady-state mass distribution and the assumed turnover rates, the postapheresis ratio of material in the two compartments and the time point when the maximal difference can be observed will differ. As long as it is assumed that LDL in compartment 2 originates from LDL in compartment 1 (Fig 1), more material will be in compartment 1 for a limited period following LDL apheresis compared with the pretreatment situation (steady state).

DISCUSSION

The aim of the present study was to evaluate in patients with heterozygous FH whether different methods of LDL apheresis affect the in vitro oxidizability of LDL and whether the pattern of plasma total fatty acids is related to the lag time before treatment and the increase in the lag phase during treatment. Using a computer simulation, we tested the hypothesis of whether an altered ratio between freshly produced (less susceptible to oxidation) and older (more susceptible to oxidation) LDL particles could explain the observed differences in the lag phase.

While the charge of apoB was not altered, we observed a significant increase in the lag phase of 9.8%. The increase was statistically significant in the immunoadsorption group; it did not reach significance in the other subgroups. A high concentration of plasma total palmitic, myristic, and oleic acid was associated with an increased pretreatment lag phase. In patients with a high concentration of lauric, linoleic, or docosahexanoic acid, we found a steeper increase in the lag phase during treatment.

The nonsignificant increase in the lag phase in the HELP and DS adsorption groups is thought to be due to the smaller sample size (HELP, n = 4; DS adsorption, n = 6). The objective of investigating different LDL procedures is that the systems used differ slightly in the elimination coefficient, which is highest and almost 1.0 in HELP. Incomplete elimination of LDL in immunoadsorption and DS adsorption could alter the posttreatment LDL oxidizability by preferential elimination of certain LDL subfractions. However, in HELP, LDL-free plasma is returned to the patient,[23] and thus, changes in LDL oxidizability during HELP cannot be due to a different elimination pattern of heterogenous LDL particles.

We have no explanation for the decrease in the lag phase in two patients on dextran sulfate apheresis. Neither had an abnormality in the fatty acid pattern. However, one patient has rheumatoid arthritis treated with nonsteroidal antiinflammatory agents. We cannot estimate whether this condition may have provoked the decrease in the lag phase with apheresis.

The determination of conjugated dienes as a measure of LDL oxidizability is an indirect method and probably does not exactly reflect in vivo LDL oxidation, yet it is regarded as the

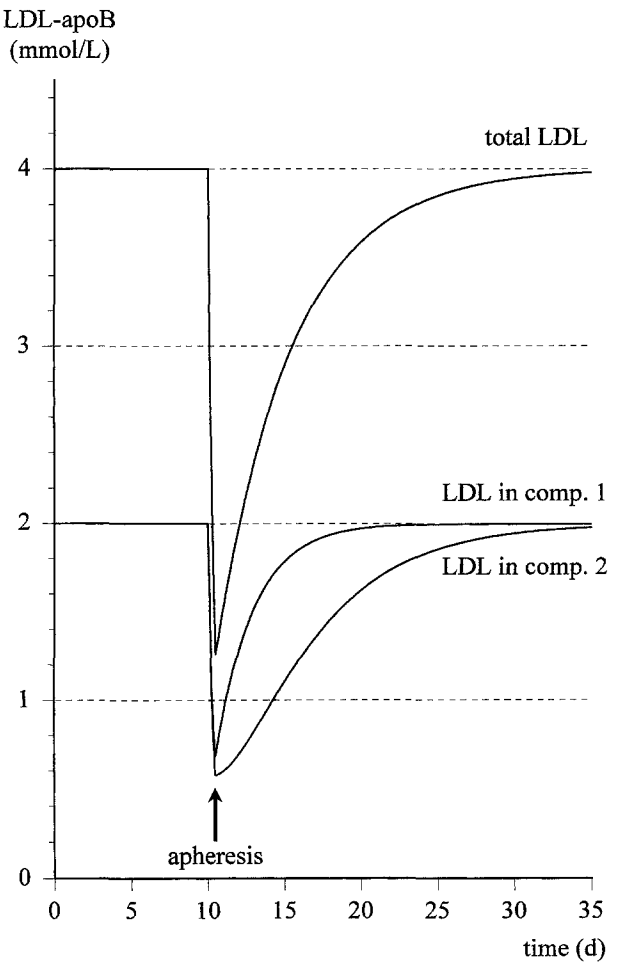

Fig 3. Simulated mass distribution between two LDL compartments following LDL apheresis. Comp., compartment.

best indirect index for LDL oxidizability.[27] Compared with supplementation with high doses of vitamin E,[28] the change in the lag phase in our study, although significant, is small. It is difficult to estimate the benefit with regard to the prevention of progression of coronary heart disease in patients with FH. However, it must be noted that LDL apheresis predominantly is a potent means for reducing LDL, thereby stabilizing coronary lesions and improving the poor prognosis in these patients. The influence on LDL oxidizability can possibly be regarded as a small additional benefit.

There have been various attempts to explain the observation of a decreased susceptibility of LDL to oxidation after apheresis. Napoli et al,[20] who used DS adsorption in eight homozygous FH patients without any lipid-towering agents, found a progressively increasing posttreatment resistance of LDL to oxidation during four treatments. They argued that exogenous removal of LDL induced changes in the chemical composition such as an increase in the LDL vitamin E and oleic acid content and a decrease in the LDL half-life and arachidonic acid content that eventually led to an increased resistance against oxidation. An increase in the vitamin E content of LDL, and additionally, plasmalogen phospholipids, was hypothesized by Leitinger et al,[21] who observed a significantly increased lag period associated with a decreased formation of thiobarbituric acid–reactive substances in six patients with heterozygous FH on regular DS adsorption.

Cattin et al[22] provide yet another explanation for the changes in oxidative parameters found in five heterozygous FH patients after one DS adsorption. On the basis of their findings of a decline of 16.7% and 54.1% in malondialdehyde equivalents in LDL and a more electronegative LDL subfraction, respectively, they conclude that predominantly older, in vivo modified LDL may be removed by LDL apheresis. However, the fact that all extracorporeal LDL is precipitated during HELP[23] suggests that there is not a selective removal of older, vitamin E–depleted LDL.

Alternately, the observed differences in the lag phase could also be the result of an altered ratio between freshly produced (less susceptible to oxidation) and older (more susceptible to oxidation) LDL particles. This was tested in a computer-simulation study. We assumed a hypothetical patient characterized by parameters typical for a patient with heterozygous FH undergoing regular LDL apheresis therapy.[29,30] LDL-apoB metabolism was described by a two-compartment model (Fig 1) in which one compartment (compartment 1) represents freshly produced LDL (less susceptible to oxidation) and the other compartment (compartment 2) represents older LDL (more susceptible to oxidation). Shortly after LDL apheresis, more LDL-apoB is in compartment 1 versus compartment 2, and thus in the compartment less susceptible to oxidation (Fig 3). While the quantitative results of the simulation study will depend on the assumptions concerning the model, the qualitative conclusions will not. As long as it is assumed that LDL in compartment 2 originates from LDL in compartment 1, more material will be in compartment 1 for a limited period following LDL apheresis compared with the pretreatment situation. If one assumes that the ratio between freshly produced and older LDL particles determines the lag phase and other parameters indicating the oxidative state of total LDL, these simulations predict a longer lag phase after LDL apheresis. Thus, the simulation indicates that the procedure itself does not alter LDL composition, but the improvement in the lag phase may simply reflect an altered ratio between freshly secreted and older LDL particles. In this context, it is noteworthy that incubation of LDL with DS in another study did not alter the susceptibility to oxidation.[31] Our hypothesis may also explain the results by Leitinger et al,[21] who observed a significant prolongation of the median posttreatment lag time in patients on regular DS adsorption. The progressively decreasing oxidizability and changing composition of LDL in the study by Napoli et al[20] is also concordant with our simulation study. When regular LDL apheresis is started in a new patient, the steady state will not be reached between the treatments and thus the imbalance between LDL in compartment 1 and LDL in compartment 2 will increase, which explains the progressively decreasing oxidizability. From our model, it can be suggested also that the decline of malondialdehyde equivalents in LDL found by Cattin et al[22] was not due to a removal of older LDL but to an unselective removal and a relative predominance of younger LDL after treatment. There are no data on long-term LDL susceptibility to oxidation, but stable vitamin E concentrations during regular immunoadsorption[18] and HELP[32] evaluated for up to 3 years suggest that the main antioxidant of LDL is not depleted during long-term LDL apheresis. Independently of the possible mechanism, our data show that the exposure of plasma to light and artificial surfaces during any of the procedures does not increase the susceptibility of LDL to oxidation.

The observed range of increase in the lag phase after treatment suggests considerable interindividual variation. The extent of the increase in the lag phase of an individual patient may be determined by other factors. The finding of a higher concentration of plasma total palmitic, myristic, and oleic acid in patients with an increased pretreatment lag phase confirms previous results. High concentrations of SFA and MuFA have been associated with a decreased susceptibility to oxidation.[9,10] The increase in the lag phase during treatment was associated with a high concentration of lauric, linoleic, and docosahexanoic acid. Whereas lauric acid, a SFA, may decrease the susceptibility to oxidation, we do not have an explanation for the positive correlation with linoleic and docosahexanoic acid. A high concentration of PuFA provides the source for lipid peroxidation and has been related to increased LDL oxidizability.[11] We suggest that there may be factors not determined in this study, such as a high content of antioxidants, that may render LDL less susceptible to oxidation and prolong the posttreatment lag period in patients with high levels of PuFA.

In conclusion, we found that LDL apheresis performed with different techniques decreases LDL susceptibility to oxidation. The simulation study indicates that this decrease may be related to a temporary mass imbalance between LDL particles characterized by different susceptibility to oxidation. Furthermore, the baseline fatty acid pattern influences pretreatment LDL oxidizability and changes in LDL oxidizability induced by apheresis.

ACKNOWLEDGMENT

The authors wish to thank E. Fleischer-Brielmaier for excellent technical assistance.

REFERENCES

1. Stary HC: Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. Arteriosclerosis 9:19-32, 1989 (suppl 1)

2. Brown MS, Goldstein J: Lipoprotein metabolism in the macrophage. Annu Rev Biochem 52:223-261, 1983

3. Lavy A, Brook GJ, Dankner G, et al: Enhanced in vitro oxidation of plasma lipoproteins derived from hypercholesterolemic patients. Metabolism 40:794-799, 1991

4. Napoli C, Postiglione A, Triggiani M, et al: Oxidative structural modification of low density lipoprotein in homozygous familial hypercholesterolemia. Atherosclerosis 118:259-273, 1995

5. Stalenhoef AF: Clinical implications of increased low-density lipoprotein oxidation in patients with hypercholesterolemia. Nutrition 10:564-566, 1994

6. Kleinveld HA, Demacker PN, De Haan AF, et al: Decreased in vitro oxidability of low-density lipoprotein in hypercholesterolaemic patients treated with 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors. Eur J Clin Invest 23:289-295, 1993

7. Esterbauer H, Dieber-Rotheneder M, Waeg G, et al: Endogenous antioxidants and lipoprotein oxidation. Biochem Soc Trans 18:1059-1061, 1990

8. Packer JE, Slater TF, Wilson RL: Direct observation of a free radical interaction between vitamin E and vitamin C. Nature 278:737-738, 1979

9. Reaven P, Parthasarathy S, Grasse B, et al: Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects. J Clin Invest 91:668-676, 1993

10. Mata P, Alonso R, Lopez-Farre A, et al: Effect of dietary fat saturation on LDL oxidation and monocyte adhesion to human endothelial cells in vitro. Arterioscler Thromb Vasc Biol 16:1347-1355, 1996

11. Parthasarathy S, Khoo JC, Miller E, et al: Low density lipoprotein rich in oleic acid is protected against oxidative modification: Implications for the dietary prevention of atherosclerosis. Proc Natl Acad Sci USA 87:3894-3898, 1990

12. Esterbauer H, Gebicki J, Puhl H, et al: The role of lipid peroxidation and antioxidants in the oxidative modification of LDL. Free Radic Biol Med 13:341-390, 1992

13. Stoffel W, Greve V, Borberg H: Application of specific extracorporeal removal of low density lipoprotein in familial hypercholesterolemia. Lancet 2:1005-1007, 1981

14. Eisenhauer T, Armstrong VW, Wieland H, et al: Selective removal of low density lipoproteins (LDL) by precipitation at low pH: First clinical application of the HELP system. Klin Wochenschr 65:161-168, 1987

15. Seidel D, Armstrong VW, Schuff-Werner P, et al: The HELP-LDL-Apheresis Multicenter Study, an angiographically assessed trial on the role of LDL-apheresis in the secondary prevention of coronary heart disease. I. Evaluation of safety and cholesterol-lowering effects during the first 12 months. Eur J Clin Invest 21:375-383, 1991

16. Homma Y, Mikami Y, Tamachi H, et al: Comparison of selectivity of LDL removal by double filtration and dextran-sulfate cellulose column plasmapheresis. Atherosclerosis 60:23-27, 1986

17. Mabuchi H, Michishita I, Takeda M, et al: A new low density lipoprotein apheresis system using two dextran sulfate cellulose columns in an automated column regenerating unit (LDL-continuous apheresis). Atherosclerosis 68:19-25, 1987

18. Richter WO, Jacob BG, Ritter MM, et al: Three-year treatment of familial heterozygous hypercholesterolemia by extracorporeal low-density lipoprotein immunoadsorption with polyclonal apolipoprotein B antibodies. Metabolism 42:888-894, 1993

19. Jacob BG, Richter WO, Schwandt P: Therapy of severe familial heterozygous hypercholesterolemia by low-density lipoprotein apheresis with immunoadsorption: Effects of the addition of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors to therapy. Clin Invest 71:908-912, 1993

20. Napoli C, Ambrosio G, Scarpato N, et al: Decreased low-density lipoprotein oxidation after repeated selective apheresis in homozygous familial hypercholesterolemia. Am Heart J 133:585-595, 1997

21. Leitinger N, Pirich C, Blazek I, et al: Decreased susceptibility of low-density lipoproteins to in-vitro oxidation after dextran-sulfate LDL-apheresis treatment. Atherosclerosis 126:305-312, 1996

22. Cattin L, Petrucco A, Cazzolato G, et al: Low density lipoprotein-apheresis decreases oxidized low density lipoproteins and monocyte adhesion to endothelial cells. ASAIO J 43:209-213, 1997

23. Richter WO, Donner MG, Schwandt P: Short- and long-term effects on serum lipoproteins of three different techniques of apheresis. Artif Organs 30:311-317, 1996

24. Esterbauer H, Striegl G, Puhl H, et al: Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radic Res Commun 6:67-75, 1989

25. Vedie B, Myara I, Pech MA, et al: Fractionation of charge-modified low density lipoproteins by fast protein liquid chromatography. J Lipid Res 32:1359-1369, 1991

26. Shimomura Y, Sugiyama S, Takamura T: Quantitative determination of fatty acid composition of human lipids by high performance liquid chromatography. J Chromatogr 383:9-17, 1986

27. Jialal I, Devaraj S: Low-density lipoprotein oxidation, antioxidants, and atherosclerosis: A clinical biochemistry perspective. Clin Chem 42:498-506, 1996

28. Jialal I, Grundy SM: Effect of combined supplementation with alpha-tocopherol, ascorbate and beta carotene on low-density lipoprotein oxidation. Circulation 88:2780-2786, 1993

29. Kesaniemi YA, Vega GL, Grundy SM: Kinetics of apolipoprotein B in normal and hyperlipidemic man: Review of the current data, in Berman M, Grundy SM, Howard BV (eds): Lipoprotein Kinetics and Modeling. New York, NY, Academic, 1982, pp 181-205

30. Parhofer GK, Barrett PHR, Demant T, et al: Effects of weekly LDL apheresis on metabolic parameters of apolipoprotein B in heterozygous familial hypercholesterolemia. J Lipid Res 37:2383-2393, 1996

31. Hermann M, Gmeiner B: Altered susceptibility to in-vitro oxidation of LDL in LDL complexes and LDL aggregates. Arterioscler Thromb 12:1503-1506, 1992

32. Armstrong VW, Niedmann D, Eisenhauer T, et al: Acute and long-term effects of low-density lipoprotein apheresis on serum concentrations of vitamins A and E. Klin Wochenschr 66:123-128, 1988