Effect of lipid transfer activity and lipolysis on low density lipoprotein (LDL) oxidizability: evidence for lipolysis-generated non-esterified fatty acids as inhibitors of LDL oxidation

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Abstract Low density lipoproteins (LDL) were modified in vitro in the presence of lipid transfer activity and lipolysis, which induced alterations in the size and lipid composition of LDL particles but not in their antioxidant content. Subsequently, modified LDL were oxidized with copper sulfate and the extent of LDL oxidation was evaluated. Lipid transfer activity alone, or in combination with lipolysis, led to a significant reduction of LDL oxidability as compared with starting homologous LDL. Furthermore, the combined effect of lipid transfers and lipolysis reduced LDL oxidability to a significantly greater extent than did lipid transfers alone. Consistent results were obtained by measuring either the formation of lipid peroxides, the appearance of thiobarbituric acid reactive substances (TBARS), the disappearance of polyunsaturated fatty acids (PUFA), or the generation of cholesterol oxides. Non-esterified fatty acids (NEFA) arose as putative candidates in reducing oxidation susceptibility of LDL: NEFA-containing LDL were less oxidizable; the enrichment of LDL with either oleic acid or linoleic acid reduced significantly their oxidability; the oxidation susceptibility of either in vitro modified LDL or LDL isolated from normal or analbuminemic patients significantly increased after reduction of their NEFA content with fatty acid-poor albumin. After NEFA depletion, small-sized LDL resulting from the combined effects of lipid transfer and triglyceride hydrolysis activities became more oxidizable than large-sized LDL treated with lipid transfer activity alone. In addition, the PUFA to total fatty acid ratio and the oxidability of modified LDL varied accordingly after NEFA depletion, showing that in the present study not only lipoprotein-bound NEFA but also the total fatty acid composition of LDL could account for alterations in their oxidability.—**Viens, L., A. Athias, G. Lizard, G. Simard, S. Gueldry, S. Braschi, P. Gambert, C. Lallemant, and L. Lagrost.** Effect of lipid transfer activity and lipolysis on low density lipoprotein (LDL) oxidizability: evidence for lipolysis-generated non-esterified fatty acids as inhibitors of LDL oxidation. *J. Lipid Res.* 1996. **37:** 2179–2192.

Supplementary key words cholesteryl ester transfer protein • lipoprotein lipase • lipid peroxides • thiobarbituric acid reactive substances • cholesterol oxides • polyunsaturated fatty acids

The structure and composition of LDL particles are susceptible to variation from one subject to another, and it has now been clearly demonstrated that plasma LDL consist of several discrete subpopulations with distinct density (1), size (2, 3), and lipid composition (1, 4). In particular, two types of plasma LDL patterns have been described on the basis of gradient gel distribution profiles (5). Pattern A is defined as an LDL pattern containing mainly large particles with minor subpopulations of smaller size; pattern B is constituted mainly of small particles with minor subpopulations of larger size (5). Based on earlier in vitro observations (6), a general model that integrates the biochemical mechanisms leading to alterations in the size distribution of LDL particles has been proposed. It involves the exchange of cholesteryl ester and triglyceride molecules between LDL and very low density lipoproteins (VLDL), mediated by the cholesteryl ester transfer protein (CETP), and the subsequent hydrolysis of triglycerides in the LDL core by endothelium lipases. Recent studies from our laboratory demonstrated further that LDL pattern A can be progressively transformed into typical LDL pattern B upon in vitro incubation of the plasma LDL fraction in the presence of lipid transfer and triglyceride hydrolysis activities (7).

Abbreviations: LDL, low density lipoproteins; VLDL, very low density lipoproteins; TBARS, thiobarbituric acid reactive substances; PUFA, polyunsaturated fatty acids; NEFA, non-esterified fatty acids; CETP, cholesteryl ester transfer protein; LPL, lipoprotein lipase; PBS, phosphate-buffered saline; BSA, bovine serum albumin; MDA, malondialdehyde; TBS, Tris-buffered saline.

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Several lines of evidence indicate that these are oxidized LDL, and not native LDL, that critically contribute to human atherosclerosis (8). Indeed, numerous studies have demonstrated that oxidatively modified LDL are recognized by scavenger receptors of macrophages and, as a consequence, can promote cellular cholesterol accumulation and foam cell formation (9). In recent reports (10–14), it was shown that the small, dense subpopulations of LDL, which predominate in human plasma with LDL pattern B, are more prone to in vitro oxidation than the larger, more buoyant subfractions. These observations suggest, therefore, that the higher risk for cardiovascular disease observed by Austin and coworkers (15) in subjects with an LDL subclass pattern B could result in part from increased concentrations of some small, highly oxidizable LDL subfractions. Given observations that small LDL are more susceptible to oxidation than large LDL, we made the hypothesis that the reduction in LDL size per se might lead to an increase in LDL oxidation susceptibility. In order to investigate the latter hypothesis, LDL particles ultracentrifugally isolated from normolipidemic human plasma were modified in vitro in the presence of lipid transfer and triglyceride hydrolysis activities according to the general procedure previously used in our laboratory (7). We report here the resulting alterations in the susceptibility to oxidation of modified LDL subfractions.

MATERIALS AND METHODS

Lipoprotein preparation

Lipoprotein fractions were isolated by sequential ultracentrifugation either from normolipidemic human plasma (total cholesterol lower than 2.5 g/L; total triglycerides lower than 1.0 g/L) or from analbuminemic plasma (16) as indicated. Densities, adjusted by the addition of potassium bromide, were checked by using a DMA 35 digital densitymeter (Paar, Graz, Austria). Very low density lipoproteins (VLDL) were isolated as the d < 1.006 g/mL plasma fraction with two successive runs, one 22-h run at 40,000 rpm (102,000 *g*) in a 65-Ti rotor on an L7 ultracentrifuge (Beckman, Palo Alto, CA), and one 2-h run at 70,000 rpm (339,000 *g*) in an NVT-90 rotor on an XL-90 ultracentrifuge (Beckman). Low density lipoproteins (LDL) were isolated as the 1.019 < d < 1.063 g/mL plasma fraction on a 70-Ti rotor on an L7 ultracentrifuge (Beckman) with one 22-h/40,000 rpm (118,000 *g*) run at the lowest density and one 22-h/45,000 rpm (149,000 *g*) run at the highest density.

Lipid transfers

The plasma LDL fraction was progressively enriched with triglycerides by incubating total plasma with freshly isolated VLDL (triglyceride concentration added, 1g/L), according to the general procedure previously described (7). Briefly, VLDL-supplemented plasma was incubated for 24 h at 37°C to allow the substitution of VLDL-derived triglycerides for cholesteryl esters in the LDL core through the neutral lipid transfer reaction mediated by the cholesteryl ester transfer protein (CETP). Plasma samples contained 1.5 mmol/L iodoacetate in order to block lecithin:cholesterol acyltransferase (LCAT) activity.

Triglyceride hydrolysis

Hydrolysis of LDL triglycerides was achieved by using lipoprotein lipase (LPL) from two distinct sources: *i)* a crude, commercially available LPL preparation which was purchased from Sigma (specific activity, 4 U/mg), and *ii)* a highly enriched LPL fraction (specific activity, 200 U/mg) which was purified from fresh bovine milk by affinity chromatography on heparin-Sepharose as described by Bengtsson and Olivecrona (17). Briefly, fresh milk was skimmed by a 1-h, 3,000 *g* centrifugation, and solid NaCl was added to make a 0.5 mol/L solution. Subsequently, 4 g of heparin-Sepharose CL 6B (Pharmacia) suspended in Tris 10 mmol/L, NaCl 0.5 mol/L, pH 7.4, was added per liter of skimmed milk. The resulting mixture was then left for 2 h at 4°C under gentle shaking. The heparin-Sepharose suspension was collected on a sintered glass filter, washed with Tris/HCl, pH 7.4, buffer containing successively 0.5 mol/L NaCl, and 0.75 mol/L NaCl. The gel was packed into a 2.5-cm diameter, 8-cm-long chromatography glass column, and elution was conducted with a 1.5 mol/L NaCl solution. Eluted active fractions were pooled, stabilized by the addition of albumin (final concentration, 10 g/L), and dialyzed against 0.5 mol/L NaCl, Tris/HCl buffer. The resulting LPL-containing fraction was applied to a second heparin-Sepharose column of smaller size (diameter, 2.5 cm; length, 4 cm). The column was washed with a 0.5 mol/L NaCl solution, and bound proteins were eluted according to a linear NaCl gradient ranging from 1 to 2 mol/L. Fractions of 3 mL were collected, and aliquots were analyzed for protein content (18), and LPL activity that was assayed according to Nilsson-Ehle and Schotz (19). Fractions with high triglyceride hydrolysis activity did not contain detectable amounts of albumin. They were stored at -80°C in 10% glycerol.

After overnight dialysis against Tris (hydroxymethyl)-aminomethane 0.2 mol/L, NaCl 0.08 mol/L, pH 8.4, buffer, LDL were lipolyzed by incubation in the presence of LPL according to the general procedure pre-

viously described (6, 7). LDL (protein concentration, 0.5 g/L), LPL (final concentration, 40 mU/mL), heparin (2 U/mL), and fatty acid-poor bovine serum albumin (60 g/L) were incubated at 37°C for 2 h (7). At the end of the incubation period, LPL activity was inhibited by the addition of KBr, which raised the final density to d = 1.07 g/mL. LDL particles were then reisolated by one 16-h run at 40,000 rpm (118,000 *g*) in a 70-Ti rotor on an L7 ultracentrifuge (Beckman).

In some experiments, isolated LDL (final protein concentration, 1.2 g/L) were incubated for one additional 2-h period at 37°C in the presence of fatty acid-poor bovine serum albumin (final concentration, 10 g/L), in order to remove further the non-esterified fatty acids that were formed in lipoprotein substrates during the LPL-mediated triglyceride hydrolysis. After that extra treatment, LDL particles were reisolated by ultracentrifugation as described above.

Enrichment of LDL with non-esterified fatty acids

Ultracentrifugally isolated LDL, diluted in a phosphate 15 mmol/L, NaCl 154 mmol/L, pH 7.4, buffer (PBS buffer) (protein concentration, 1.4 g/L), were incubated for 90 min at 37°C in the presence of either oleic acid or linoleic acid (final concentrations, 250 or 500 μmol/L) that were added as concentrated ethanolic solutions (20). Control LDL without fatty acid supplementation received only ethanol. LDL fractions were subsequently dialyzed overnight against PBS buffer.

LDL oxidation

LDL were oxidized by incubation in the presence of copper sulfate. Briefly, isolated LDL were diluted in PBS (final protein concentration, 0.5 g/L), and incubated for 11 or 24 h at 37°C in the presence of a copper sulfate solution (final concentration, 2 μmol/L) according to the general methodology previously described (21). Samples were removed at selected times and the reaction was stopped by the addition of 200 μmol/L of ethylenediamine-tetraacetic acid (EDTA).

Measurement of lipid peroxides

Lipid peroxides were measured by the spectrophotometric method described by El Saadani et al. (22), adapted on a COBAS-BIO centrifugal analyzer (Roche, Basel, Switzerland). This test is based on the capacity of lipid peroxides to convert iodide to triiodide, which can be measured by photometry. Briefly, 10 μL of LDL (protein concentration, 0.5 g/L) reacted for 30 min at room temperature with 100 μL of reagent, and the absorbance was measured at 365 nm. The concentration of lipid peroxides in LDL samples was calculated by using the molar extinction of triiodide ($\varepsilon = 2.46\ 10^4\ M^{-1} \cdot cm^{-1}$) (22).

Assay of the thiobarbituric acid reactive substances (TBARS)

TBARS were determined in LDL samples by using a spectrophotometric method derived from the thiobarbituric assay described by Buege and Aust (23). Briefly, 1 mL of a reagent containing thiobarbituric acid (0.375%) and trichloroacetic acid (15%) was added to 500 μL of an LDL solution (protein concentration, 0.5 g/L). After 15 min of incubation at 90°C the mixture was cooled and centrifuged at low temperature on an Eppendorf centrifuge for 5 min. The absorbance of the supernatant was determined immediately at 535 nm by using a Gilford 250 spectrophotometer (Oberlin, Ohio). The results were expressed as malondialdehyde equivalents per gram of LDL protein (μmol MDA/g), using a molar extinction of $1.56\ 10^5\ M^{-1} \cdot cm^{-1}$ (23).

Assay of oxidized cholesterol derivatives

Cholesterol oxides in LDL were analyzed by capillary gas chromatography (24) on a Hewlett-Packard 5890 gas chromatograph attached to a 5971A mass detector (Hewlett-Packard, Palo Alto, CA). After addition of epicoprostanol as an internal standard, total lipids were extracted from LDL by the method of Folch, Lees, and Sloane Stanley (25), saponified for 60 min at 60°C with potassium hydroxide, and silylated by incubation at 80°C for 60 min with a mixture containing N,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA):trimethylchlorosilane (5TMCS) (4:1). Samples were injected onto a Hewlett-Packard 12.5-m-long methylsilicone fused silica column by using a 7673A auto-injector (Hewlett-Packard). The temperature of the injector and mass detector was 250°C and 280°C, respectively. Temperature was set at 150°C for 3 min and then programmed to reach 220°C at the rate of 5°C/min and 280°C at the rate of 10°C/min; helium pressure was 35 kPa.

The ions at *m/z* 368, 370, 472, 384, and 456 were used to follow the trimethylsilyl ether derivative of cholesterol, epicoprostanol, 7-ketocholesterol, 5α,6α-epoxycholesterol, and both 7α-hydroxycholesterol, 7β-hydroxycholesterol, respectively. Concentrations of cholesterol and cholesterol oxides were determined from the ratio of the peak area corresponding to one given molecule to the peak area corresponding to the internal standard.

Determination of the fatty acid composition of LDL

Total fatty acids in LDL were analyzed by capillary gas chromatography (26) on a Hewlett-Packard 5890 gas chromatograph attached to a 5971A mass detector

(Hewlett-Packard, Palo Alto, CA). Total lipids from LDL were extracted by the method of Folch et al. (25). The extract was saponified at 60°C for 60 min with potassium hydroxide (13.2 g/l) followed by esterification at 60°C for 60 min with boron trifluoride (BF3)-methanol to give fatty acid methyl esters. The chromatographic analysis was as described above for oxidized cholesterol derivatives, except for the oven temperature which increased from 220°C up to 280°C at a rate of 10°C/min. Heptadecanoic acid (17:0) was added as an internal standard to each sample before extraction and the fatty acid contents were determined from the ratio of the peak area of the sample to the peak area of the internal standard.

Analysis of antioxidant content of LDL

α-Tocopherol, γ-tocopherol, α-carotene, and β-carotene were assayed by high performance liquid chromatography (HPLC) according to the general method described by Miller and Yang (27). Briefly, LDL lipids were extracted in darkness with an ethanol–hexane solution 1:3. The hexane fraction was evaporated under nitrogen and reconstituted in a mixture of acetonitril–methanol 60:25. The chromatographic analysis was performed by using a Gold HPLC System (Beckman), on a 220 × 4.6 mm Spheri-5 RP 18 column (Brownlee) which was connected to a Diode Assay Detector 168. δ-Tocopherol was added to each sample as an internal standard prior to the extraction and the antioxidant content was determined from the ratio of the peak area of the sample to the peak area of the internal standard. The antioxidant content values in isolated LDL were in the same range as previously reported (28).

Electrophoretic analysis of LDL subfractions

The mean apparent diameter of plasma LDL particles was determined by nondenaturating electrophoresis in 20–160 g/L polyacrylamide gradient gels (PAA 2/16; Pharmacia, Uppsala, Sweden) (29). The electrophoresis conditions were as previously described (7). At the end of the electrophoresis, the gels were fixed, stained with Coomassie brilliant blue G, and destained (30). The distribution profile of LDL was finally obtained by densitometric scanning of the gels at 640 nm with a Bio-Rad GS-670 densitometer. The apparent diameter of the predominant LDL subfraction was determined by comparison with ferritin (diameter, 12.20 nm; Pharmacia), thyroglobulin (diameter, 17.00 nm; Pharmacia), and carboxylated latex beads (diameter, 38.00 nm; Duke Scientific) that were subjected to electrophoresis with the LDL samples.

Other lipid analyses

All chemical assays were performed on a COBAS-BIO centrifugal analyzer (Roche, Basel, Switzerland). Protein concentration was measured by using bicinchoninic acid reagent (Pierce) according to the method of Smith et al. (31). Total cholesterol, unesterified cholesterol, phopholipids, and triglycerides were measured by enzymatic methods using Boehringer Mannheim reagents. Non-esterified fatty acids were assayed by using a commercially available enzymatic kit (Wako Pure Chemical Industries, Osaka, Japan).

Statistical analysis

The significance of the difference between data means was determined by using either Wilcoxon signed-

TABLE 1. Effect of lipid transfers on the size and lipid composition of plasma LDL particles

	Control	Transfer
Size of the major LDL subfraction, nm	25.4 ± 0.4	25.7 ± 0.4[a]
Lipid content		
UC[b]	0.53 ± 0.14	0.45 ± 0.16[a]
CE[b]	1.90 ± 0.50	1.49 ± 0.50[a]
PL[b]	0.99 ± 0.17	0.91 ± 0.22
TG[b]	0.29 ± 0.08	0.71 ± 0.10[a]
NEFA[c]	7.9 ± 7.8	11.9 ± 10.5[a]
Fatty acid composition[d]		
16:0	286 ± 71	358 ± 107[a]
16:1	32 ± 11	45 ± 13[a]
18:0	115 ± 23	133 ± 32
18:1	255 ± 38	346 ± 46[a]
18:2	453 ± 73	439 ± 66
20:4	80 ± 23	76 ± 21
Total PUFA/total FA	0.44 ± 0.05	0.38 ± 0.06[a]

Plasma samples from normolipidemic subjects were supplemented with a lecithin:cholesterol acyltransferase inhibitor (iodoacetate, 1.5 mmol/L) and were either maintained at 4°C (Control) or incubated for 24 h at 37°C with VLDL supplementation (final concentration of added triglyceride, 1 g/L) (Transfer). Subsequently, LDL were isolated by ultracentrifugation and their size, lipid content, and fatty acid composition were determined as described in Materials and Methods. Values of the size, lipid content, and fatty acid composition of LDL are mean ± SD of four, six, and six similar experiments, respectively. Each experiment was performed in triplicate. PUFA, polyunsaturated fatty acids; FA, fatty acids.

[a] $P < 0.05$ versus homologous Control samples (Wilcoxon signed-rank test).

[b] Unesterified cholesterol (EC), cholesteryl ester (CE), phospholipid (PL), and triglyceride (TG) contents are expressed as g/g LDL protein.

[c] Nonesterified fatty acid (NEFA) content is expressed as μmol/g LDL protein.

[d] Palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and arachidonic acid (20:4) contents are expressed as mg/g LDL protein.

rank test, Mann-Whitney test, or ANOVA, as indicated. Coefficients of correlation, ρ, were calculated by using Spearman rank correlation analysis.

RESULTS

Effect of lipid transfers on the size and composition of LDL particles

The lipid composition of plasma LDL particles was modified by lipid transfer activity in the presence of added, exogenous VLDL (see Materials and Methods). As shown in **Table 1,** a slight but significant increase in the mean size of the major LDL subfraction was observed in incubated plasmas (Transfer samples) compared with control homologues maintained at 4°C (Control samples). Lipid transfer activity induced a significant decrease in both unesterified cholesterol/protein and cholesteryl ester/protein mass ratios, while the phospholipid/protein mass ratio was unaffected (Table 1). In contrast, the triglyceride and nonesterified fatty acid (NEFA) contents of LDL were significantly increased when total human plasma was incubated in the presence of VLDL. In fact, the replacement of cholesteryl esters by triglycerides appeared as one of the most striking changes in the composition of LDL particles. The composition of total fatty acids was significantly modified by the lipid transfer reaction which induced significant increases in the palmitic, palmitoleic, and oleic acid contents of LDL (Table 1). No change or even a slight reduction in the linoleic and arachidonic acid contents of Transfer LDL was observed (Table 1). As a consequence, the PUFA to total fatty acid mass ratio was significantly lower in Transfer than in Control LDL (Table 1).

As assessed by using high performance liquid chromatography, changes in the size and lipid composition of LDL particles were not accompanied by significant alterations in the detectable antioxidative components (α-tocopherol, 13.65 ± 2.27 μmol/g of LDL protein in Control vs. 13.22 ± 0.45 μmol/g of LDL protein in Transfer, n = 3, n.s.; γ-tocopherol, 1.97 ± 0.09 μmol/g of LDL protein in Control vs. 1.80 ± 0.15 μmol/g of LDL protein in Transfer, n = 3, n.s.; α-carotene, 3.07 ± 0.94 μmol/g of LDL protein in Control vs. 2.21 ± 0.16 μmol/g of LDL protein in Transfer, n = 3, n.s.; β-carotene, 6.87 ± 1.27 μmol/g of LDL protein in Control vs. 5.06 ± 0.66 μmol/g of LDL protein in Transfer, n = 3, n.s.) (Mann-Whitney test).

Effect of lipid transfers on the oxidability of LDL particles

In order to determine whether changes in the structure and composition of LDL were accompanied by alterations in their oxidation susceptibility, LDL fractions isolated from Control and Transfer plasma samples were incubated for 0, 11, or 24 h in the presence of copper sulfate, as described under Materials and Methods. At the end of the incubation period, the extent of LDL oxidation was evaluated by assessing various oxidation criteria, namely the formation of lipid peroxides, thiobarbituric acid reactive substances (TBARS), and cholesterol oxides, as well as the disappearance of unsaturated fatty acids.

Time course of the formation of lipid peroxides and TBARS in LDL during copper-mediated oxidation is

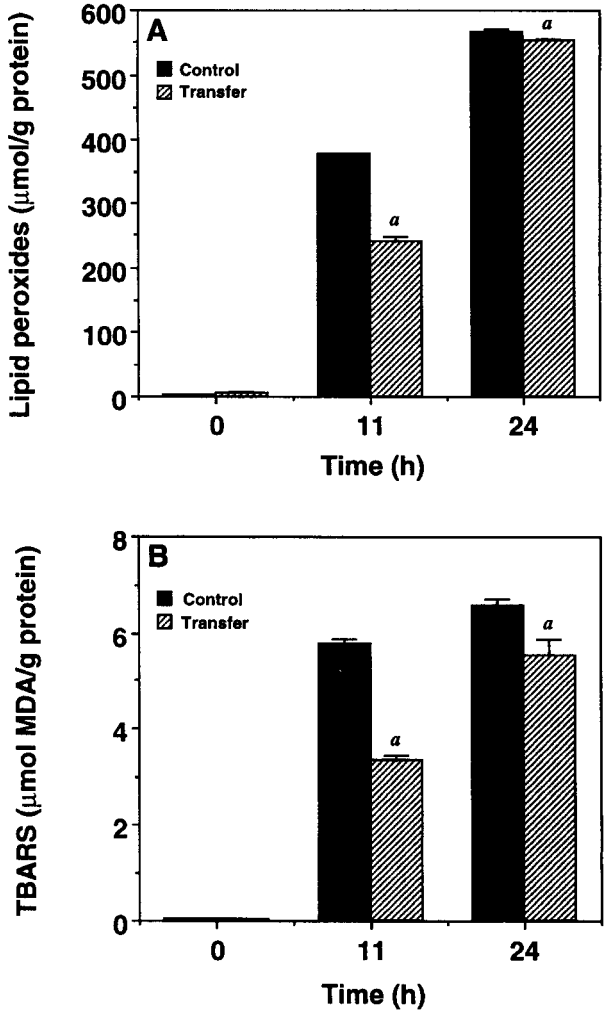

Fig. 1. Effect of lipid transfers on the formation of lipid peroxides and thiobarbituric acid reactive substances (TBARS) in oxidized LDL. LDL isolated from incubated (Transfer samples) or non-incubated (Control samples) plasma (see Table 1) were incubated with copper sulfate for 11 and 24 h at 37°C. Lipid peroxides (panel A) and thiobarbituric acid reactive substances (TBARS) (panel B) were assayed as described under Materials and Methods. Data are means ± SD of triplicate determinations and are representative of four similar experiments. Data means were compared by using Mann-Whitney test. $^{a}P < 0.05$ vs. homologous Control sample.

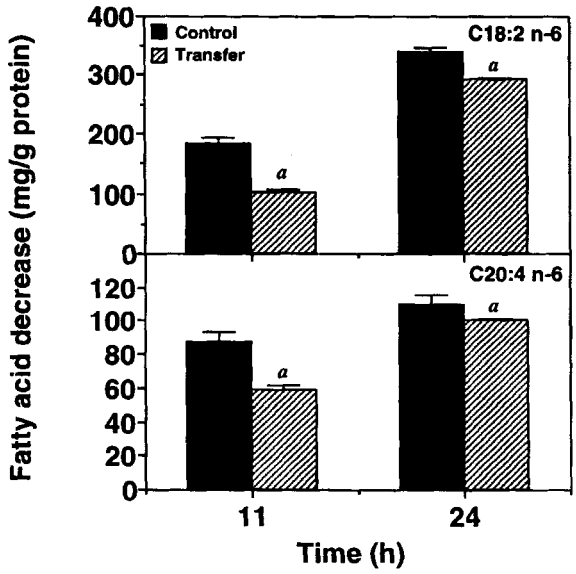

Fig. 2. Effect of lipid transfers and oxidation on the polyunsaturated fatty acid content of LDL. LDL isolated from incubated (Transfer samples) or non-incubated (Control samples) plasma (see Table 1) were incubated with copper sulfate for 11 and 24 h at 37°C. Linoleic acid (C18:2 n-6) and arachidonic acid (C20:4 n-6) contents were measured as described under Materials and Methods. Data, corresponding to fatty acid decrease as compared with non-oxidized homologues, are means ± SD of triplicate determinations and are representative of four similar experiments. Data means were compared by using Mann-Whitney test. $^{a}P < 0.05$ vs. homologous Control sample.

shown in **Fig. 1.** Significant levels of both oxidation products were detected in LDL that were oxidized for 11 or 24 h in the presence of copper sulfate. The formation of lipid peroxides and TBARS was significantly lower in LDL that were previously modified by the lipid transfer process (Transfer LDL) than in Control LDL. In accordance with the lower production of oxidized fatty acid in Transfer LDL than in Control LDL, the mean net decrease in the polyunsaturated fatty acid (linoleic and arachidonic acids) content of LDL was significantly lower in incubated samples (**Fig. 2**). In accordance with previous studies (32, 33), detectable amounts of oxygenated derivatives of cholesterol, i.e., 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol, were generated in LDL in a time-dependent manner during oxidation in the presence of copper sulfate. As shown in **Fig. 3,** the amount of cholesterol oxides formed during LDL oxidation was significantly lower in Transfer LDL than in Control LDL. Consistent observations were made after 11 or 24 h of incubation.

Taken together, the observations described above indicate that the effect of lipid transfer activity, which leads in vitro to the formation of large-sized, triglyceride-enriched LDL, results in a significant reduction of the LDL oxidability.

Combined effects of lipid transfers and triglyceride hydrolysis on the size and composition of LDL particles

LDL particles were successively submitted to lipid transfer activity and to triglyceride hydrolysis catalyzed by the commercially available lipoprotein lipase (LPL) fraction (see Materials and Methods). The size and composition of LDL particles resulting from that sequential treatment are presented in **Table 2.** As compared with non-lipolyzed LDL (Transfer, -LPL samples), incubation in the presence of LPL (Transfer, +LPL samples) induced a marked, significant reduction in the triglyceride content of LDL (Table 2). In addition, a slight but significant, reduction in the phospholipid content of LDL was also observed (Table 2). The amount of non-esterified fatty acid, one of the main end products of lipolysis, was markedly increased in LPL-treated LDL. In contrast, neither unesterified cholesterol nor cholesteryl ester contents of LDL were affected by the

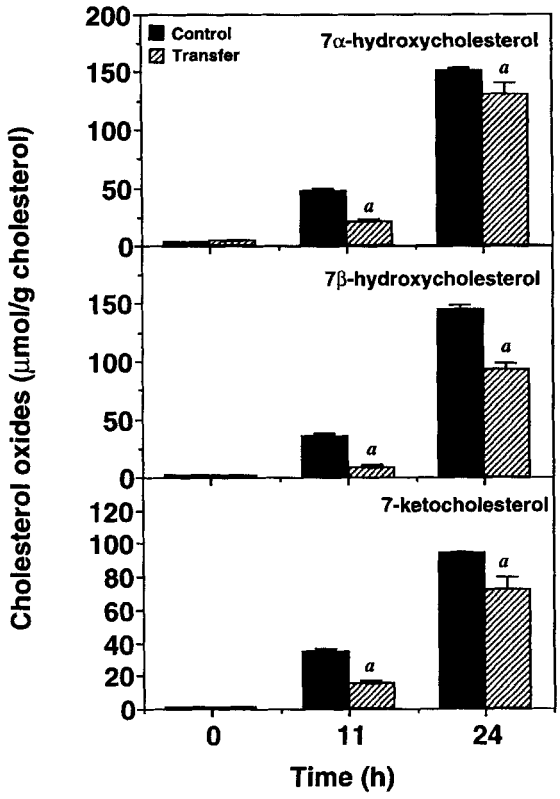

Fig. 3. Effect of lipid transfers on the formation of cholesterol oxides in oxidized LDL. LDL isolated from incubated (Transfer samples) or non-incubated (Control samples) plasma (see Table 1) were incubated with copper sulfate for 11 and 24 h at 37°C. 7α-Hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol were measured as described under Materials and Methods. Data are means ± SD of triplicate determinations and are representative of four similar experiments. Data means were compared by using Mann-Whitney test. $^{a}P < 0.05$ vs. homologous Control sample.

TABLE 2. Effect of lipid transfers and lipolysis on the size and lipid composition of plasma LDL particles

	Transfer, -LPL	Transfer, +LPL
Size of the major LDL subfraction, nm	25.6 ± 0.4	24.8 ± 0.7[a]
Lipid content		
UC[b]	0.46 ± 0.09	0.47 ± 0.13
CE[b]	1.25 ± 0.27	1.23 ± 0.31
PL[b]	0.73 ± 0.19	0.63 ± 0.23[a]
TG[b]	0.72 ± 0.10	0.27 ± 0.03[a]
NEFA[c]	12.4 ± 10.6	28.0 ± 14.4[a]
Fatty acid composition[d]		
16:0	367 ± 111	246 ± 65[a]
16:1	46 ± 13	26 ± 8[a]
18:0	131 ± 34	103 ± 23[a]
18:1	350 ± 56	211 ± 41[a]
18:2	428 ± 72	336 ± 76[a]
20:4	77 ± 22	65 ± 26[a]
Total PUFA/total FA	0.37 ± 0.05	0.41 ± 0.07[a]

Plasma samples from normolipidemic subjects were supplemented with a lecithin:cholesterol acyltransferase inhibitor (iodoacetate, 1.5 mmol/L) and were incubated for 24 h at 37°C with VLDL supplementation (final concentration of added triglyceride, 1 g/L). Subsequently LDL were isolated by ultracentrifugation, dialyzed against TBS, pH 8.4, and incubated for 2 h at 37°C in the absence (Transfer, -LPL) or in the presence (Transfer, +LPL) of commercially available bovine milk lipoprotein lipase (LPL) (10 μg/mL; specific activity, 4 U/mg). The size, lipid content, and fatty acid composition of LDL were determined as described in Materials and Methods. Values of the size, lipid content, and fatty acid composition of LDL are mean ± SD of four, five, or five similar experiments, respectively. Each experiment was performed in triplicate. PUFA, polyunsaturated fatty acids; FA, fatty acids.

[a]$P < 0.05$ versus homologous Transfer, -LPL samples (Wilcoxon signed-rank test).

[b]Unesterified cholesterol (UC), cholesteryl ester (CE), phospholipid (PL), and triglyceride (TG) contents are expressed as g/g LDL protein.

[c]Nonesterified fatty acid (NEFA) expressed as μmol/g LDL protein.

[d]Contents expressed as mg/g LDL protein.

LPL treatment. The LPL-mediated lipolysis of LDL significantly reduced the total fatty acid content of LDL, and the PUFA to total fatty acid mass ratio was significantly higher in lipolyzed LDL than in lipid transfer-treated counterparts (Table 2).

The levels of antioxidant components that were detected in lipolyzed LDL were not significantly altered when compared with non-lipolyzed analogs (α-tocopherol, 10.76 ± 0.48 μmol/g of LDL protein in Transfer, -LPL vs. 9.95 ± 0.49 μmol/g of LDL protein in Transfer, +LPL, n = 3, n.s.; γ-tocopherol, 1.93 ± 0.01 μmol/g of LDL protein in Transfer, -LPL vs. 1.87 ± 0.05 μmol/g of LDL protein in Transfer, +LPL, n = 3, n.s.; β-carotene, 0.98 ± 0.13 μmol/g of LDL protein in Transfer, -LPL vs. 0.97 ± 0.08 μmol/g of LDL protein in Transfer, +LPL, n = 3, n.s.) (Mann-Whitney test).

Alterations in the lipid composition of LDL particles induced by the combined effects of neutral lipid transfers and lipolysis were associated with a significant reduction in their mean apparent diameter (Table 2). More specifically, the mean apparent diameter of the major LDL subfraction decreased from 25.6 nm, one characteristic of LDL pattern A (mean diameter greater than 25.5 nm), to 24.8 nm, one characteristic of LDL pattern B (mean diameter lower than 25.5 nm) (5).

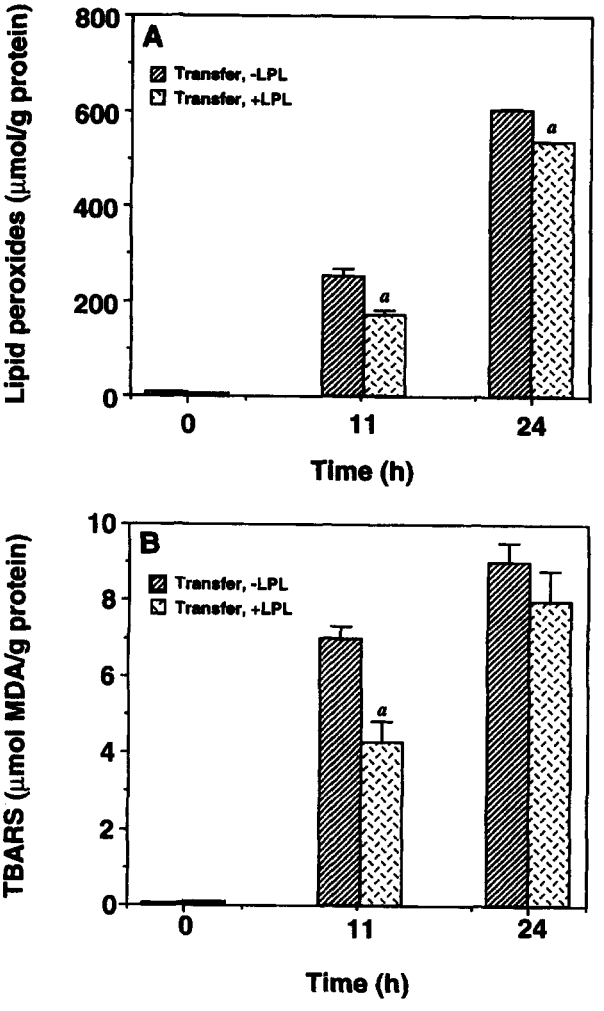

Fig. 4. Effect of lipid transfers and lipolysis on the formation of lipid peroxides and thiobarbituric acid reactive substances (TBARS) in oxidized LDL. LDL isolated from incubated plasma were subjected (Transfer, +LPL samples), or not (Transfer, -LPL samples), to triglyceride hydrolysis catalyzed by commercially available lipoprotein lipase (LPL). Subsequently, Transfer, -LPL and Transfer, +LPL LDL samples were incubated with copper sulfate for 11 and 24 h at 37°C. Lipid peroxides (panel A) and thiobarbituric acid reactive substances (TBARS) (panel B) were assayed as described under Materials and Methods. Data are means ± SD of triplicate determinations and are representative of four similar experiments. Data means were compared by using Mann-Whitney test. [a]$P < 0.05$ vs. homologous Transfer, -LPL sample.

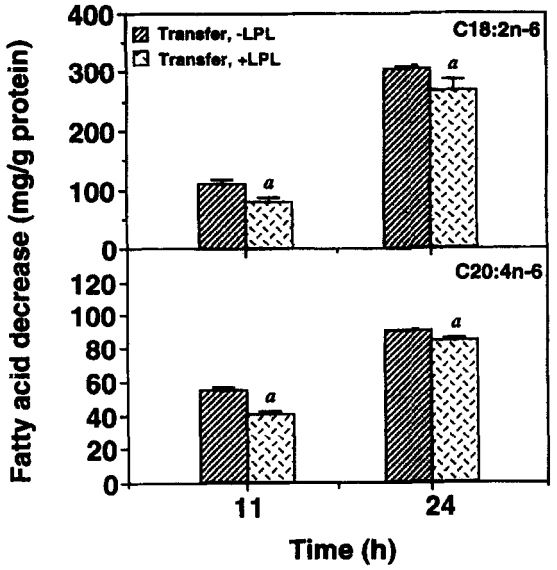

Fig. 5. Effect of lipid transfers, lipolysis, and oxidation on the polyunsaturated fatty acid content of LDL. LDL isolated from incubated plasma were subjected (Transfer, +LPL samples), or not (Transfer, -LPL samples), to triglyceride hydrolysis catalyzed by commercially available lipoprotein lipase (LPL). Subsequently, Transfer, -LPL and Transfer, +LPL LDL samples were incubated with copper sulfate for 11 and 24 h at 37°C. Linoleic acid (C18:2 n-6) and arachidonic acid (C20:4 n-6) contents were measured as described under Materials and Methods. Data, corresponding to fatty acid decrease as compared with non-oxidized homologues, are means ±SD of triplicate determinations and are representative of four similar experiments. Data means were compared by using Mann-Whitney test. $^{a}P < 0.05$ vs. homologous Transfer, -LPL sample.

Combined effects of lipid transfers and triglyceride hydrolysis on the oxidability of LDL particles

To investigate the consequences of the combination of lipid transfers and lipolysis in terms of LDL oxidability, LDL isolated from Transfer, -LPL and Transfer, +LPL samples were subjected to an oxidative stress in the presence of copper sulfate, according to the general procedure described above. The formation of both lipid peroxides (**Fig. 4A**) and TBARS (Fig. 4B) was significantly lower in the lipolyzed LDL than in non-lipolyzed homologues. Consistent observations were made after 11 and 24 h of incubation. In support of a reduced oxidability of lipolyzed LDL, the decrease in the polyunsaturated fatty acid (linoleic acid and arachidonic acid) content of LDL was significantly less pronounced in Transfer, +LPL samples than in Transfer, -LPL homologues (**Fig. 5**). Furthermore, detectable cholesterol oxides, i.e., 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol, were produced in lower amounts in lipolyzed than in non-lipolyzed LDL (**Fig. 6**).

In summary, it results that the combined effects of lipid transfer activity and lipolysis, which lead in vitro to the formation of small-sized, core-depleted, and NEFA-enriched LDL particles, are associated with a significant reduction in the oxidability of LDL. Consistent observations were made by using either the commercially available LPL fraction or the highly enriched LPL fraction (results not shown).

Effect of non-esterified fatty acid depletion of LDL particles on their oxidability

In a first attempt to study the role of NEFA in reducing LDL oxidability, plasma LDL isolated from several normolipidemic subjects were preincubated or not with fatty acid-poor bovine serum albumin, a process which is known to reduce the NEFA content of lipoprotein particles (34, 35). Indeed, LDL preincubated with BSA contained significantly lower NEFA levels than untreated LDL (5.8 ± 5.6 vs. 20.5 ± 6.4 μmol/g of LDL protein, respectively; n = 7; $P < 0.0001$; Wilcoxon signed-rank test). The decrease in the NEFA content of LDL was accompanied by a significant increase in their

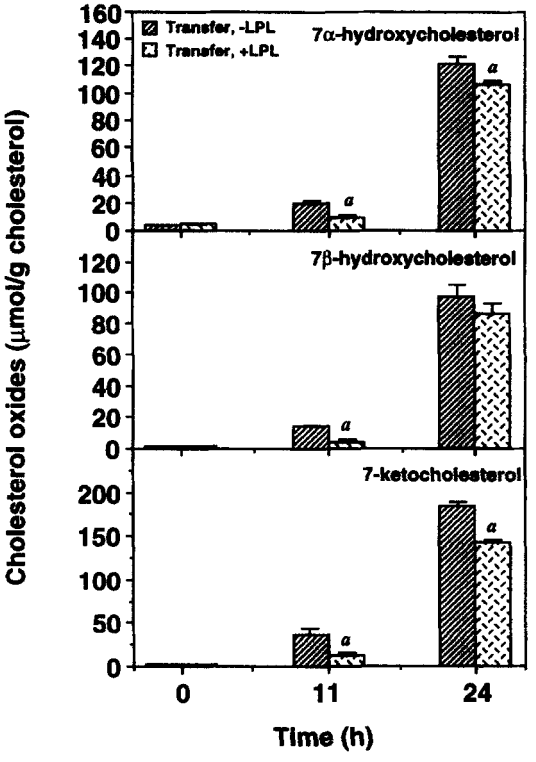

Fig. 6. Effect of lipid transfers and lipolysis on the formation of cholesterol oxides in oxidized LDL. LDL isolated from incubated plasma were subjected (Transfer, +LPL samples), or not (Transfer, -LPL samples), to triglyceride hydrolysis catalyzed by commercially available lipoprotein lipase (LPL). Subsequently, Transfer, -LPL and Transfer, +LPL LDL samples were incubated with copper sulfate for 11 and 24 h at 37°C. 7α-Hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol were measured as described under Materials and Methods. Data are means ± SD of triplicate determinations and are representative of four similar experiments. Data means were compared by using Mann-Whitney test. $^{a}P < 0.05$ vs. homologous Transfer, -LPL sample.

TABLE 3. Effect of nonesterified fatty acid depletion on the oxidizability of LDL from analbuminemic patients

	NEFA		Lipid Peroxides	
	-BSA	+BSA	-BSA	+BSA
	μmol/g protein		*μmol/g protein*	
ZA	81 ± 2	10 ± 1[a]	496 ± 6	648 ± 12[a] (+30.6%)
BR	38 ± 3	7 ± 1[a]	601 ± 10	726 ± 20[a] (+20.8%)
RR	50 ± 3	8 ± 1[a]	546 ± 7	675 ± 13[a] (+23.6%)

The plasma LDL fraction from three analbuminemic patients (ZA, BR, RR) was isolated as described in Materials and Methods. Subsequently, LDL were incubated or not for 2 h at 37°C in the presence of fatty acid-poor BSA (30 g/L) prior to being oxidized with copper sulfate for 11 h at 37°C. NEFA and lipid peroxides were assayed as described in Materials and Methods. Data are mean ± SD of triplicate determinations. Numbers in parentheses indicate the percentage difference from homologous samples without BSA treatment. Statistical analyses were performed on triplicate determinations obtained with each patient.

[a]$P < 0.05$ versus homologous -BSA samples (Mann-Whitney).

oxidability as higher amounts of copper-generated lipid peroxides were measured in BSA-treated LDL than in untreated counterparts after an 11-h oxidation period (578 ± 139 vs. 546 ± 131 μmol/g of LDL protein; n = 7; $P < 0.03$; Wilcoxon signed-rank test).

A similar experimental approach was applied to plasma LDL that were ultracentrifugally isolated from patients with analbuminemia, an inherited disease with abnormally low plasma albumin concentrations (16). As shown in **Table 3,** and in accordance with previous observations from our laboratory (16), the NEFA content of analbuminemic LDL before BSA treatment was clearly higher than that measured in normal LDL. Interestingly, the BSA-mediated decrease in the NEFA content of analbuminemic LDL was accompanied by a dramatic increase in the generation of lipid peroxides (Table 3).

The role of lipolysis-generated NEFA in reducing LDL oxidation was further investigated by incubating LDL isolated from the Control, Transfer, -LPL and Transfer, +LPL samples for 2 h at 37°C with fatty acid-poor BSA. Subsequently, LDL were reisolated by sequential ultracentrifugation (see Materials and Methods) and analyzed for their size, lipid content, and oxidability. Alterations in the size, unesterified cholesterol content, cholesteryl ester content, triglyceride content, and phospholipid content of BSA-treated LDL were in conformity with data obtained under identical conditions, but with no extra BSA treatment (results not shown). After BSA treatment, LDL became significantly more prone to oxidation than untreated homologues, and consistent observations were made by measuring lipid peroxides (**Fig. 7**), and cholesterol oxides (**Fig. 8**). Whereas LPL-treated LDL remained less oxidizable than control LDL, they became more oxidizable than Transfer, -LPL counterparts after BSA treatment, as observed by measuring the formation of lipid peroxides (Fig. 7), and cholesterol oxides (Fig. 8) ($P < 0.01$ in all cases; ANOVA). Among the various experimental conditions studied (see Figs. 7 and 8), the NEFA content of LDL correlated negatively with the formation of both cholesterol oxides ($\rho = 0.94$; $P < 0.03$; n = 6), and lipid peroxides ($\rho = 0.94$; $P < 0.03$; n = 6) (**Fig. 9**).

Effect of NEFA supplementation on LDL oxidability

To investigate further the hypothesis of a putative role of lipoprotein-bound NEFA in limiting LDL oxidation, LDL isolated from normolipidemic subjects were supplemented, or not, with oleic acid prior to be subjected to an oxidative stress in the presence of copper sulfate. Briefly, LDL were preincubated for 90 min at 37°C in

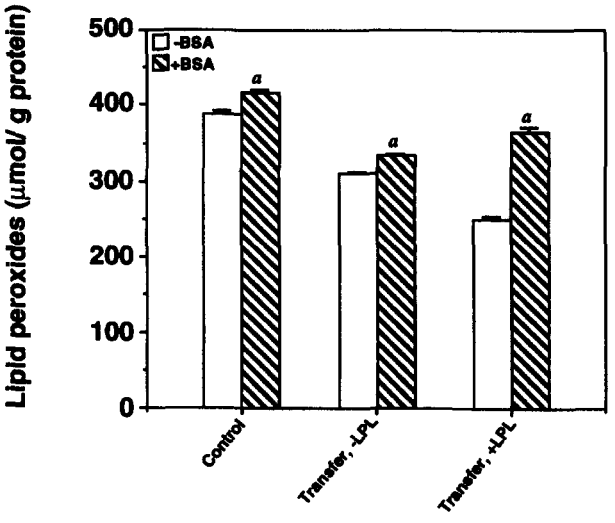

Fig. 7. Effect of fatty acid-poor albumin on the formation of lipid peroxides in oxidized LDL. LDL isolated from the Control, Transfer, -LPL, and Transfer, +LPL samples described above were incubated (+BSA) or not (-BSA) with bovine serum albumin (final concentration, 10 g/L) for 2 h at 37°C and were then oxidized with copper sulfate for 11 h at 37°C (see Materials and Methods). Lipid peroxides were measured as described under Materials and Methods. Data are means ± SD of triplicate determinations. Data means were compared by using Mann-Whitney test. [a]$P < 0.05$ vs. homologous -BSA sample.

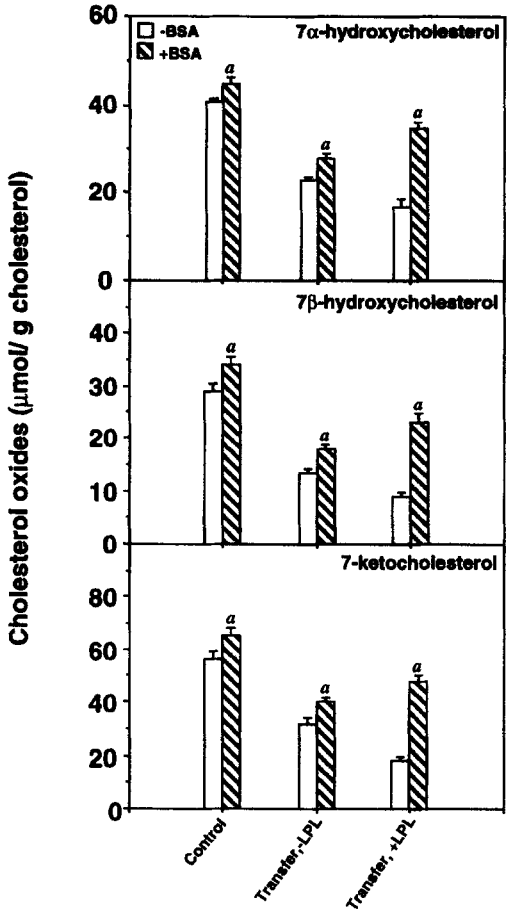

Fig. 8. Effect of fatty acid-poor albumin on the formation of cholesterol oxides in oxidized LDL. LDL isolated from the Control, Transfer, -LPL, and Transfer, +LPL samples described above were incubated (+BSA) or not (-BSA) with bovine serum albumin (final concentration, 10 g/L) for 2 h at 37°C and were then oxidized with copper sulfate for 11 h at 37°C (see Materials and Methods). 7α-Hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol were measured as described under Materials and Methods. Data are means ±SD of triplicate determinations. Data means were compared by using Mann-Whitney test. $^{a}P < 0.05$ vs. homologous -BSA sample.

the absence or in the presence of oleic acid at a concentration of 250 or 500 μmol/L (see Materials and Methods). At the end of the preincubation period, LDL were dialyzed overnight against PBS buffer prior to be incubated for 11 h at 37°C in the presence of copper sulfate. During the 90-min, 37°C preincubation of LDL, a fraction of oleic acid added became incorporated into the LDL structure. The final NEFA/protein ratio in LDL that were preincubated with 250 and 500 μmol/L of oleic acid were 155 ± 3 and 238 ± 2 μmol/g of LDL protein, respectively. **Table 4** shows the effect of oleic acid incorporation on the copper-induced generation of lipid peroxides and TBARS. After 11 h of oxidation, the amount of lipid peroxides and TBARS formed was significantly lower in the oleic acid-supplemented LDL than in non-supplemented counterparts. Moreover, the amount of lipid peroxides formed was significantly lower in LDL preincubated with 500 μmol/L of oleic acid than in LDL preincubated with only 250 μmol/L of oleic acid. In contrast, the substantial reduction in TBARS did not vary significantly with the dose of oleic acid added.

In order to determine whether the degree of unsaturation of NEFA could affect their ability to reduce LDL oxidability, LDL supplemented with either oleic acid (NEFA content, 138 μmol/g of LDL protein), or linoleic acid (NEFA content, 146 μmol/g of LDL protein) were oxidized with copper sulfate. The levels of lipid peroxides in oleic acid-supplemented LDL (277 ± 5 μmol/g LDL protein), and in linoleic acid-supplemented LDL (340 ± 4 μmol/g LDL protein) were significantly lower than in non-supplemented control LDL (388 ± 5 μmol/g LDL protein) ($P < 0.0001$; ANOVA). Interestingly, the level of lipid peroxides was significantly greater in linoleic acid-supplemented LDL than in oleic acid-supplemented LDL ($P < 0.0001$; ANOVA), indicating that non-esterified linoleic acid is less effective than non-esterified oleic acid in reducing LDL oxidability.

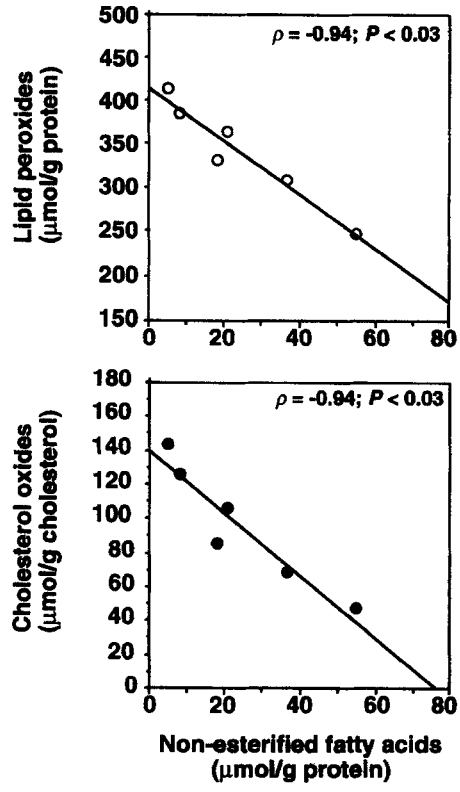

Fig. 9. Correlations between the NEFA content and the oxidability of LDL. LDL were treated as described in Fig. 7 and 8 and their cholesterol oxide, lipid peroxide, and NEFA contents were determined as described under Materials and Methods. Correlations were analyzed by using Spearman rank correlation analysis.

TABLE 4. Effect of oleic acid supplementation on the formation of lipid peroxides and thiobarbituric acid reactive substances (TBARS) in oxidized LDL

	Lipid Peroxides		TBARS	
	0 h	11 h	0 h	11 h
	μmol/g protein		*μmol MDA/g protein*	
Control LDL	6 ± 1	651 ± 8	0.11 ± 0.02	12.85 ± 0.31
LDLA	5 ± 0.4	591 ± 10[a]	0.12 ± 0.03	10.70 ± 0.50[a]
LDLB	10 ± 0.1	536 ± 45[a,b]	0.11 ± 0.02	10.81 ± 0.07[a]

LDL isolated form normolipidemic plasma were incubated with oleic acid (final concentration added to LDLA and LDLB, 250 and 500 μmol/L, respectively) for 90 min at 37°C as described in Materials and Methods. LDL were dialyzed against PBS and incubated with copper sulfate (final concentration, 2 μmol/L) for 11 h at 37°C. Lipid peroxides and thiobarbituric acid reactive substances (TBARS) were determined as described in Materials and Methods. Values are mean ± SD of one representative experiment performed in triplicate and are expressed as μmol malondialdehyde (MDA) equivalent per gram of LDL protein and μmol of lipid peroxides per gram of LDL protein for TBARS and lipid peroxide measurements, respectively. LDLA and LDLB fractions contained 155 ± 3 and 238 ± 2 μmol of NEFA per gram of LDL protein, respectively. Control LDL did not contain detectable amounts of NEFA. Data means were compared using ANOVA.

[a]$P < 0.01$ versus homologous Control LDL.

[b]$P < 0.01$ versus homologous LDLA.

DISCUSSION

Whereas the underlying mechanisms that account for the association of the small-sized LDL pattern B with coronary artery disease (CAD) have not yet been fully elucidated, recent studies suggested that they may relate in some way to the significantly higher oxidability of the small LDL particles as compared with the larger ones (10–14). Several studies suggested that reduced resistance to oxidation of small-sized LDL may relate either to their decreased antioxidant content (12, 13), to their reduced unesterified cholesterol content (11), to their high PUFA content (10), or to their elevated triglyceride content (36). In the present study, in vitro oxidation of LDL was achieved by incubation of LDL with copper sulfate. Although the oxidation of LDL with copper sulfate is nonphysiological in nature, due to the sequestration in vivo of metal ions by proteins, it is highly reproducible (37, 38) and presents the advantage of producing oxidized LDL with many structural and functional features of LDL modified by cells (39) or extracted from arterial atherosclerotic plaques (40). In order to evaluate the extent of LDL oxidation, we selected several methods corresponding roughly to different stages of the LDL oxidation cascade. Thus, the degree of LDL oxidation was evaluated by the direct measurement of generated lipid peroxides, the detection of TBARS, the disappearance of unsaturated fatty acids, as well as the generation of oxygenated cholesterol derivatives.

Isolated LDL were subjected successively to lipid transfer activity and lipolysis (6, 7), an experimental approach that has the advantage of modifying the size and lipid composition of LDL particles without inducing significant changes in their antioxidant content, a parameter that may account for alteration in the oxidability of distinct plasma LDL subfractions independently of their overall structure and lipid composition (12, 13). In accordance with previous observations (6, 7), lipid transfer activity alone tended to increase the mean size of LDL particles and induced the replacement of cholesteryl esters by triglycerides in the LDL core. In contrast, the combination of lipid transfer activity and LPL-mediated lipolysis was characterized mainly by decrease in the size and triglyceride content of LDL as compared with non-lipolyzed homologues (6, 7). As observed by determining LDL mean diameter on polyacrylamide gradient gels, and in accordance with a recent study from our laboratory (7), the combined effects of lipid transfers and lipolysis could promote the transformation of typical LDL pattern A into typical LDL pattern B. In addition to previous studies, the present work revealed that the sole action of lipid transfer activity, as well as the combined effect of lipid transfer activity and LPL, are associated with significant rise in the non-esterified fatty acid (NEFA) content of LDL particles and with variations in the relative composition of the total fatty acid moiety.

Lipid transfers alone or in combination with lipolytic activity led to a significant reduction of LDL oxidability as compared with starting homologous material. Furthermore, the combined effect of lipid transfers and lipolysis reduced LDL oxidability to a greater extent than did lipid transfers alone. Consistent data were obtained by measuring the formation of lipid peroxides, TBARS, and cholesterol oxides, as well as the disappearance of unsaturated fatty acids. Lipid transfer activity alone and the combination of lipid transfer activity and lipolysis produced no effect on the antioxidant content of LDL. In contrast, the two treatments produced oppo-

site effects on the size and on the neutral lipid content of LDL. It is therefore rather unlikely that under those experimental conditions, variation in either the size or the neutral lipid content of LDL may have constituted the major determinants of LDL oxidability, as proposed previously by others in plasma LDL fractions (10–14).

The NEFA content of LDL and their oxidability varied in an inverted manner under the various experimental conditions used in the present study. Hence, the NEFA content of LDL emerges in the present study as one putative candidate in reducing LDL oxidability. In support of this latter view, reducing the NEFA content of LDL particles by preincubation of LDL with fatty acid-poor albumin significantly increased their oxidability and the NEFA content and oxidability of LDL were shown to correlate negatively. In particular, the BSA treatment induced a dramatic increase in the oxidability of NEFA-rich LDL from analbuminemic patients, suggesting that the inhibition of LDL oxidation by NEFA might be of physiological relevance at least under pathological conditions that are associated with elevated NEFA contents of plasma lipoproteins, i.e., analbuminemia and nephrotic syndrome (16, 34). In addition, the enrichment of isolated LDL with non-esterified fatty acids was shown to reduce significantly their susceptibility to undergo oxidative modifications. The inhibitory effect of lipoprotein-bound NEFA can be related to the resistance to iron-induced lipid peroxidation observed in intestinal mucosal membranes (41), a model that is characterized by an elevated NEFA content (42). Whereas it is still unclear whether NEFA may have affected LDL oxidability by influencing the particle surface charge, and thus possibly Cu^{2+}-LDL interactions or by some other direct effect on Cu^{2+}, the ability of NEFA to reduce LDL oxidability appeared to be dependent on the degree of unsaturation of their acyl carbon chain. Indeed, non-esterified oleic acid was a more potent inhibitor of LDL oxidation than non-esterified linoleic acid. Significant differences in the oxidation susceptibility between LDL fractions were still apparent when the NEFA contents of lipolyzed LDL and triglyceride-enriched, non-lipolyzed LDL were brought to similar, low levels by using fatty acid-poor albumin. However, it is noteworthy that the lipolyzed, small-sized, and NEFA-depleted LDL became more oxidizable than the non-lipolyzed, large-sized, and NEFA-depleted counterparts. Therefore, unlike data obtained with NEFA-containing modified LDL, the latter observations support the previous studies that demonstrated that small-sized LDL are more prone to oxidation than LDL particles of large size (10–14). They suggest that in the absence of lipoprotein-bound NEFA other factors are responsible for alterations in LDL oxidability, and the higher oxidability of triglyceride-rich, small-sized LDL observed in vivo might relate to several independent factors, among them the antioxidant content of the various plasma LDL subfractions (12, 13).

The total fatty acid composition of LDL, in particular their relative content in polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids, may also constitute another source of oxidation variability (43–47). The decrease in PUFA content of LDL, and in particular the replacement of PUFA by monounsaturated fatty acids, has been shown to be associated with a reduced susceptibility of LDL to oxidation (43–47). In the present study, lipid transfers, which led to the replacement of LDL cholesteryl esters by VLDL triglycerides, were associated with significant increases in the palmitic, palmitoleic, and oleic acid contents of LDL, but with no change, or even a slight reduction, in their linoleic and arachidonic acid contents. Overall, the PUFA to total fatty acid mass ratio was significantly higher in non-modified than in lipid transfer-modified LDL. Differences in the PUFA to total fatty acid ratio between non-modified and lipid transfer-modified LDL might relate, in fact, to differences in the fatty acid composition of plasma cholesteryl esters and triglycerides. Indeed, it is well known that the proportion of linoleic and arachidonic acid residues in plasma cholesteryl esters is clearly higher than that observed in plasma triglycerides (48). Conversely, triglycerides are relatively enriched with saturated and monounsaturated fatty acid residues (48). As observed in the present study, it results, therefore, that the reduction of the PUFA to total fatty acid ratio in LDL, relating to the replacement of cholesteryl esters by triglycerides, might account at least in part for the lower oxidability of lipid transfer-modified LDL as compared with non-modified counterparts. On the other hand, the PUFA to total fatty acid mass ratio was significantly higher in lipolyzed LDL than in lipid transfer-modified, non-lipolyzed homologues. Therefore, the higher PUFA to total fatty acid mass ratio might account for the higher oxidability of NEFA-depleted, lipolyzed LDL as compared with the NEFA-depleted, triglyceride-rich, and non-lipolyzed homologues. These latter observations indicate that the high triglyceride content of LDL per se may not account directly for the previously reported association between high plasma triglyceride levels and elevated LDL oxidability (36).

In conclusion, lipid transfer activity alone or in combination with lipolysis can induce marked changes in the oxidability of LDL particles, independent of their antioxidant content. In fact, the alterations in LDL oxidability observed in the present study were explained mainly in terms of NEFA content and total fatty acid composition. Whether differences observed after exposure of

isolated LDL to remodeling events may account for differences in oxidative behavior among plasma LDL subfractions deserves further investigations.

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