Oxidative modification of low density lipoprotein in normal and hyperlipidemic patients: effect of lysophosphatidylcholine composition on vascular relaxation

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Abstract The elevated level of plasma low density lipoprotein (LDL) in hyperlipidemic patients is an important risk factor for the production of atherosclerosis. Plasma LDL must be modified before it can produce an impairment of endothelium-dependent relaxation in aortic rings or enhancement of uptake by macrophages. The dramatic increase in lysophosphatidylcholine (lysoPC) content in oxidatively modified LDL has been touted as an important biochemical factor for the impairment of endothelium-dependent relaxation. The present study was designed to examine the lysoPC composition of oxidized LDL samples from normal and hyperlipidemic subjects, and their effects on the impairment of endothelium-dependent relaxation. Oxidatively modified LDL from hyperlipidemic patients contained a slightly higher level (17%) of lysoPC, but produced a disproportionately greater impairment of endothelium-dependent relaxation than that from normal subjects. As lysoPC is composed of many molecular species, its composition in oxidized LDL samples was analyzed. In hyperlipidemic patients, lysoPC samples were found to contain a higher proportion of long-chain acyl groups. Subsequent studies revealed that only long-chain lysoPC ($C_{\geq 16:0}$) were effective in impairing endothelium-dependent relaxation. Experimental loading of oxidized LDL from normal subjects with long chain lysoPC to mimic levels observed in oxidized LDL from hyperlipidemic patients resulted in further impairment of endothelium-dependent relaxation. We conclude that the greater proportion of long-chain lysoPC found in the oxidized LDL of hyperlipidemic subjects is responsible for the increased impairment of endothelium-dependent vascular relaxation. We propose that the high level of LDL found in the plasma of hyperlipidemic patients, coupled with its enhanced ability to generate long chain species of lysoPC during oxidative modification, are important factors for the development of atherosclerosis in these patients.—**Chen, L., B. Liang, D. E. Froese, S. Liu, J. T. Wong, K. Tran, G. M. Hatch, D. Mymin, E. A. Kroeger, R. Y. K. Man, and P. C. Choy.** Oxidative modification of low density lipoprotein in normal and hyperlipidemic patients: effect of lysophosphatidylcholine composition on vascular relaxation. *J. Lipid Res.* 1997. **38:** 546–553

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Low density lipoprotein (LDL) is the main source of blood cholesterol (1). In hyperlipidemic patients, plasma levels of LDL are associated with the occurrence of atherosclerosis (2) and impairment of endothelial function in arteries (3–5). It is generally accepted that atherosclerotic lesions are initiated by an enhancement of LDL uptake by monocytes and macrophages. Events leading to the production of atherosclerosis include the attraction of monocytes to the arterial wall, their transformation sequentially into macrophages and foam cells, and the subsequent immobilization of macrophages and foam cells in the arterial wall. This effect is potentiated by injury to endothelial and smooth muscle cells (6). While the transformation of the LDL-laden macrophages into foam cells and the role of cholesterol in this transformation have been demonstrated, the mode of enhanced LDL uptake by the macrophages has not been clearly defined. In the liver, the uptake of plasma LDL is mediated via specific LDL receptors (1); but a scavenger receptor system is used by macrophages (7, 8). Studies on the uptake of LDL in macrophages have demonstrated that plasma LDL must be modified prior to uptake. Examples of such modification include the acetylation of LDL (9), the in vitro oxidation of LDL in the presence of transitional metals (10), and other modifications of LDL by cell-mediated biochemical processes (11–13). The presence of oxidized LDL in atherosclerotic lesions (14, 15) supports the notion that LDL oxidation is indeed an initial step in the development of atherosclerosis (16).

There are several experimental processes for the pro-

Abbreviations: PC, phosphatidylcholine; lysoPC, lysophosphatidylcholine; LDL, low density lipoprotein.

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duction of oxidized LDL, including the oxidative modification by $CuSO_4$. The effects of oxidation include the loss of endogenous antioxidants, changes in electrophoretic mobility of LDL, increases in lipid peroxides and the formation of lysophosphatidylcholine (lysoPC) (17, 18). The oxidatively modified LDL has been shown to affect cellular functions associated with the regulation of vascular tone (19, 20), activation of inflammatory responses (21–23), and platelet aggregation (24, 25). Although lipid peroxidation products (thiobarbituric acid reactive substances, TBARS) are often used to quantify the degree of oxidation in LDL (26), such analysis does not necessarily reflect the change in its atherogenic properties (27). A bioassay that has proven useful in the latter context concerns the effect of oxidized LDL on endothelium-dependent relaxation in aortic rings (27). The elevated level of lysoPC in oxidatively modified LDL has been shown to be a biochemical factor related to the impairment of endothelium-dependent relaxation in aortic ring preparations from rats, rabbits, cattle, and pigs (20, 27–30). Alternatively, high concentrations of native LDL may have direct inhibitory effects on endothelium-dependent relaxation in rabbit aorta (31).

LysoPC in LDL is not a single homogenous entity, but a combination of molecular species with different acyl groups. The level of lysoPC is normally low in native LDL, but becomes significantly higher in oxidized LDL (23, 27). If lysoPC is an important factor for the production of atherosclerotic lesions, its level and/or composition must be altered in the LDL of hyperlipidemic patients after oxidative modification. In this study, the content and composition of lysoPC in LDL from normal and hyperlipidemic patients were determined before and after oxidation. The abilities of various molecular species of lysoPC, both directly and as incorporated into LDL, to impair the endothelium-dependent relaxation of aortic ring preparations were investigated.

MATERIALS AND METHODS

Materials

Phenylephrine hydrochloride was obtained from the pharmacy of the Health Sciences Centre, Winnipeg, Canada. Reagents for lipid phosphorus determination, EDTA, and acetylcholine were purchased from the Sigma Chemical Company, St. Louis, MO. Molecular species of lysoPC were obtained from Serdary Company, London, Ontario. Thin-layer chromatographic plates (G-25) were the product of Macherey-Nagel and purchased through Brinkmann Instruments, Rexdale, Canada. BF_3 methanol (12% w/w) was obtained from Supelco Canada Ltd., Oakville, Ontario. All other chemicals were of reagent grade and were obtained through the Canlab Division of Travenol Canada Inc., Mississauga, Ontario. Mature male Sprague-Dawley rats, 250 ± 50 g were obtained from Charles River Canada Inc., St. Constante, Quebec.

Isolation of LDL and preparation of oxidatively modified LDL

Blood samples were obtained after informed consent from normal healthy volunteers of both sexes (total cholesterol < 5.2 mmol/l) and from hyperlipidemic patients (total cholesterol > 6.2 mmol/l, enrolled at the Lipid Clinic, Health Sciences Centre, Winnipeg) after overnight fasting. The age range of normal volunteers was between 25 and 50 years and the age range of hyperlipidemic patients was between 30 and 65 years. None of the hyperlipidemic patients was under active treatment at the time of blood collection. Blood samples were collected in EDTA-containing tubes (4 mM) and concentrations of total serum cholesterol, high density lipoprotein (HDL)-cholesterol, and triglyceride were determined by enzymatic analysis using commercial test kits (Boehringer Mannheim, Mannheim, Germany). LDL-cholesterol was calculated by the Friedewald equation (32). LDL (d 1.019–1.063 g/ml) was isolated from freshly prepared plasma by sequential ultracentrifugation (33). EDTA (0.3 mM) was placed in all buffers in order to prevent autooxidation of the lipoproteins. The LDL sample was dialyzed for 24 h at 4°C against three changes of phosphate-buffered saline (composition: NaCl 140 mM, KCl 3mM, Na_2HPO_4 8 mM and KH_2PO_4 2 mM) prior to use. In order to enable independent observations, LDL samples from different individuals were not pooled.

Subsequent to isolation, LDL samples were dialyzed against an EDTA-free phosphate-buffered saline. In a typical experiment, aliquots of LDL samples (containing 1.0 mg LDL protein/ml) were treated with 5 μM $CuSO_4$ at 37°C for 24 h with constant agitation to initiate oxidation. The duration of incubation and the concentration of the $CuSO_4$ used in this study have been optimized previously (27). LDL samples were used for determination of lipid contents and the molecular composition of lysoPC before and after oxidation.

Biochemical analysis of native and oxidized LDL

The protein concentration in the LDL sample was determined by the method of Lowry et al. (34). Lipids in the LDL samples were extracted by a chloroform–methanol mixture 2:1 (v/v), and the total lipid phosphorus in each sample was determined by the method of Bartlett (35). The lipid extract was separated into phospholipid groups by thin-layer chromatography with a sol-

vent system of chloroform–methanol–acetic acid–water 70:30:2:4 (v/v). Phospholipid fractions on the thin-layer chromatographic plate were visualized by iodine vapor and the lipid phosphorus content in each fraction was determined by the method of Bartlett (35).

Determination of acyl groups in lysoPC

The acyl content in the lysoPC sample was determined by gas–liquid chromatography. The acyl groups in the sample were converted into methyl esters in the presence of boron trifluoride (36). Briefly, the solvent in the sample was evaporated under a stream of nitrogen, and 0.5 ml of BF_3 reagent was added to the dried sample. Methylation of the sample took place in a screw-capped tube which was heated at 96°C for 5 min. Subsequent to heating, 0.5 ml water and 1.5 ml petroleum ether were added to the reaction mixture. The methyl esters formed were recovered in the petroleum ether phase and the efficiency of methylation was over 95%. The methyl esters were analyzed by a Shimadzu Mini-2 gas–liquid chromatograph (Tekscience; Oakville, Ontario, Canada) equipped with 15% DEGS (on 80/100 Chromosorb W/AW) columns. Heptadecanoic acid methyl ester was used as a standard for quantitation. The data obtained were analyzed by a Shimadzu Chromatopac CR601 integrator.

Loading of native LDL with lyso-PC

Native LDL was incubated with 0.5 mM 1-lauroyl-*sn*-glycero-3-phosphocholine ($C_{12:0}$-lysoPC), 1-myristoyl-*sn*-glycero-3-phosphocholine ($C_{14:0}$-lysoPC), or 1-palmitoyl-*sn*-glycero-3-phosphocholine ($C_{16:0}$-lysoPC) for 24 h at room temperature. The LDL mixture was then dialyzed for 24 h with three changes of buffer to remove the free lysoPC in the solution (27). The amount of lysoPC incorporated into the LDL sample was determined prior to use.

Incorporation of lyso-PC species into oxidized LDL

Modification of the lysoPC composition in oxidized LDL was carried out by incubation of the oxidized lipoprotein with exogenous lysoPC species. The lysoPC content of the oxidized LDL was determined, and 3.5 ml of LDL (1 mg protein/ml) was incubated with a small amount (up to 0.25 μmol) of 1-palmitoyl-*sn*-glycero-3-phosphocholine ($C_{16:0}$-lysoPC) at room temperature for 24 h. The incubating mixture was dialyzed for 24 h with three changes of buffer to remove the free lysoPC in the solution as described in our previous report (27). LysoPC contents in the dialysate as well as in the LDL were determined. Approximately 90% of the exogenous lysoPC was found to be associated with the oxidized LDL.

Assessment of endothelium-dependent relaxation in aortic rings

After intraperitoneal administration of an anticoagulant and sedative (800 units of heparin and 0.65 mg diazepam, respectively), rats were killed by cervical dislocation. The thoracic aorta was removed and each aortic ring (3 mm segment) was suspended isometrically between two horizontal steel wires in an organ bath. A resting tension of 2.0 g was applied to the aortic ring and changes in tension were measured with a Grass FT03C force-displacement transducer. The organ bath contained 10 ml of Krebs-Henseleit buffer which was maintained at 37°C and saturated with a 95% O_2–5% CO_2 gas mixture. After an equilibration period of 90 min, the aortic ring was precontracted with phenylephrine (10^{-6} M). When the contraction had become stable, acetylcholine (10^{-8}–10^{-5} M) was added cumulatively to produce endothelium-dependent relaxation. The maximum relaxation produced by acetylcholine at each concentration was expressed as a percentage of the phenylephrine-induced contraction. Criteria for acceptability of the endothelium/vascular ring preparation included active contraction of at least 1.5 g and acetylcholine-induced relaxation of 80%. After the washout of phenylephrine and acetylcholine, the preparation was incubated with oxidized LDL (0.3 mg LDL protein per ml) for 1 h. The oxidized LDL was removed from the bath prior to the determination of contraction by phenylephrine and relaxation by acetylcholine. The removal of LDL from the bath was necessary as the presence of LDL has been shown to inactivate the endothelium-derived relaxing factor. For comparison, native LDL and/or no addition were used as controls in separate experiments. When muscles were exposed to lysoPC, the latter was added to the bath 15 min prior to the first addition of Ach and remained in the bath throughout the exposure to Ach.

Statistical analyses

Student's *t* test for paired and unpaired data was used where appropriate. For multiple groups, analysis of variance (ANOVA) was used followed by the Student-Neuman-Keuls test to detect individual differences. Data are presented as mean ± standard error of the mean (SEM) and a P value < 0.05 was considered to be statistically significant.

RESULTS

The cholesterol and triglyceride concentrations in blood samples of normal individuals were determined and compared with those obtained from hyperlipid-

emic patients. The levels of total cholesterol, LDL cholesterol, and triglyceride were significantly elevated in the plasma of the hyperlipidemic patients (**Table 1**). The amount of LDL estimated from LDL protein was also elevated in these patients. The data confirmed the hyperlipidemic status of these patients. The values obtained from normal individuals were within the desirable range defined by the Canadian Cholesterol Consensus Guidelines.

The ability of oxidized LDL from hyperlipidemic patients to impair the endothelium-dependent relaxation of aortic rings was investigated. Untreated (native) LDL preparations obtained from normal individuals and hyperlipidemic patients were not significantly different in their effects on endothelium-dependent relaxation of the aortic ring (**Fig. 1**). Oxidized LDL from both groups caused greater impairment of endothelium-dependent relaxation. The oxidized LDL samples obtained from hyperlipidemic patients, however, were significantly more effective (at equal concentrations) in impairing the endothelium-dependent relaxation than those obtained from normal individuals.

The biochemical change leading to the greater impairment of endothelium-dependent relaxation by oxidized LDL from hyperlipidemic patients was examined. Lipids were extracted from native and oxidized LDL preparations from both subject groups, and the content of each phospholipid class was analyzed by thin-layer chromatography. No significant differences in the total phospholipid or sphingomyelin contents were detected in the native LDL preparations of either subject group (**Table 2**). After oxidation, the total phospholipid and sphingomyelin contents in LDL were not significantly changed in the two groups. A substantial decrease (45%) in the level of phosphatidylcholine (PC) and a concomitant increase in the level of lysoPC were detected. There was no significant difference in the PC levels between both subject groups, but the level of lysoPC was slightly higher (17%) in the hyperlipidemic group.

TABLE 1. Plasma lipid and lipoprotein contents in normal and hyperlipidemic subjects

	Normal	Hyperlipidemic
	μmol/ml plasma	
Total cholesterol	4.4 ± 0.6	7.2 ± 0.9
LDL cholesterol	2.6 ± 0.5	4.7 ± 0.8
HDL cholesterol	1.4 ± 0.2	1.2 ± 0.2
Triglyceride	1.2 ± 0.3	2.7 ± 1.0
LDL protein (μg/ml)	0.6 ± 0.2	1.1 ± 0.1

Blood samples for analysis were taken from healthy volunteers (normal, age range: 25–50 years) and patients attending a local lipid clinic (hyperlipidemic, age range: 30–65 years, not under active treatment at time of blood collection). Results represent the means ± SEM for 24 subjects in each group. Means for data from hyperlipidemic patients are significantly ($P < 0.05$) different from normal subjects in all categories except HDL cholesterol.

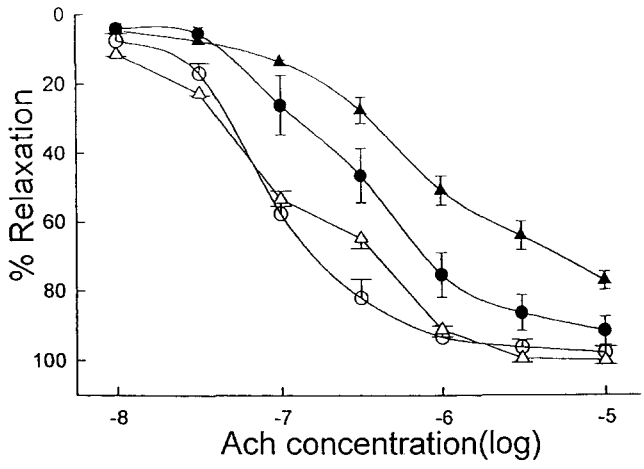

Fig. 1. Effect of low density lipoprotein (LDL) on the impairment of endothemium-dependent relaxation of the rat aortic ring. The aortic ring preparation was exposed to unoxidized (open symbols) or oxidized LDL (0.3 mg/ml, closed symbols) from normal (○, ●) or hyperlipidemic (△, ▲) subjects for 1 h. Subsequently, the aortic ring preparation was washed with buffer and the acetylcholine-induced endothelium-dependent relaxation was determined. Each point represents the mean of at least eight separate experiments. The vertical bar represents the SEM.

The generation of lysoPC in LDL upon oxidation has been regarded as a biochemical factor in the enhanced impairment of endothelium-dependent relaxation (27). In view of the enhanced impairment of endothelium-dependent relaxation caused by oxidized LDL from hyperlipidemic patients, we expected a much higher level of lysoPC in these LDL samples. As the latter expectation was not met, our next approach was to determine the acyl profiles of lysoPC in the oxidized LDL from the two subject groups. The lysoPC fraction was isolated from the LDL preparation and the acyl composition was analyzed by gas–liquid chromatography. A lower percentage of short- and medium-chain ($C_{<16}$) acyl groups was detected in the hyperlipidemic subjects when compared with the normal group. An increase in the proportion of long-chain ($C_{\geq 16}$) acyl groups, however, was observed in both subject groups after oxidative modification (**Table 3**). The net content of long-chain lysoPC in the oxidized LDL from hyperlipidemic patients was found to be 42% higher (calculated from Tables 2 and 3; $P < 0.05$) than that from normal subjects. Interestingly, there is no significant change in the percentage of long chain acyl groups in PC from oxidized LDL of the control (74 ± 3) and hyperlipidemic (76 ± 4) subjects.

Changes in the acyl composition could provide a biochemical mechanism for the differential endothelium-related effects of the oxidized LDL from hyperlipidemic

TABLE 2. Phospholipid contents in native and oxidized LDL from normal and hyperlipidemic patients

Phospholipid	Normal		Hyperlipidemic	
	Native	Oxidized	Native	Oxidized
	μmol/mg LDL protein			
Phosphatidylcholine	0.85 ± 0.02	0.46 ± 0.04[a]	0.81 ± 0.04	0.43 ± 0.05[a]
Lysophosphatidylcholine (LysoPC)	0.05 ± 0.01	0.34 ± 0.02[ab]	0.06 ± 0.02	0.40 ± 0.03[ab]
Sphingomyelin	0.26 ± 0.03	0.27 ± 0.03	0.27 ± 0.02	0.28 ± 0.03
Total phospholipid	1.31 ± 0.08	1.20 ± 0.09	1.34 ± 0.11	1.27 ± 0.09

Phospholipids were extracted from the native and oxidized LDL samples (described in Table 1) and separated by thin-layer chromatography. Results represent the means ± SEM for 24 subjects in each group.

[a]Indicates that mean values of the phospholipids are different ($P < 0.05$) between native and oxidized LDL.

[b]Indicates that mean values obtained from oxidized LDL are different ($P < 0.05$) between normal and hyperlipidemic subjects.

and normal subjects. In evaluating this possibility, the direct effect of medium- (C_{12-14}) and long-chain (C_{16-18}) lysoPC on the impairment of endothelium-dependent relaxation was investigated. The presence of 10 μM lauroyl-lysoPC ($C_{12:0}$) and myristoyl-lysoPC ($C_{14:0}$) did not significantly impair the endothelium-dependent relaxation of the aortic ring whereas 10 μM palmitoyl-lysoPC ($C_{16:0}$) caused a strong impairment of that relaxation (**Fig. 2**). The impairment of endothelium-dependent relaxation produced by stearoyl-lysoPC ($C_{18:0}$) or oleoyl-lysoPC ($C_{18:1}$) is similar to that produced by palmitoyl-lysoPC (data not shown).

TABLE 3. Distribution (%) of acyl groups in lysophosphatidylcholine in native and oxidized LDL from normal and hyperlipidemic patients

Acyl Group	Normal		Hyperlipidemic	
	Native	Oxidized	Native	Oxidized
$C_{\leq 10}$	9 ± 2	9 ± 2	2 ± 0.5	1 ± 0.5
$C_{12:0}$	5 ± 1	3 ± 1	2 ± 1	1 ± 0.5
$C_{12:1}$	6 ± 1	5 ± 2	2 ± 0.5	<1
$C_{14:0}$	4 ± 0.5	4 ± 1	6 ± 2	3 ± 0.5
$C_{14:1}$	6 ± 1	5 ± 1	3 ± 0.5	1 ± 0.5
$C_{16:0}$	23 ± 2	26 ± 3	27 ± 3	35 ± 4
$C_{16:1}$	10 ± 2	9 ± 2	8 ± 2	7 ± 2
$C_{18:0}$	14 ± 2	18 ± 3	17 ± 2	23 ± 3
$C_{18:1}$	7 ± 2	7 ± 1	17 ± 3	17 ± 3
$C_{18:2}$	3 ± 1	4 ± 1	2 ± 0.5	3 ± 1
$C_{18:3}$	4 ± 1	5 ± 2	5 ± 2	4 ± 1
$C_{20:4}$	5 ± 1	5 ± 1	7 ± 2	6 ± 2
Others	4 ± 2	6 ± 3	2 ± 1	1 ± 0.5
Short and medium chain ($C_{<16:0}$)	30 ± 2	22 ± 2[a]	15 ± 2[b]	6 ± 2[a,b]
Long chain ($C_{\geq 16:0}$)	70 ± 2	78 ± 3[a]	85 ± 3[b]	94 ± 3[a,b]

Each set of data represents the mean ± SEM of 15 subjects randomly selected within the two groups as described in Table 1. There was no age-related difference in fatty acyl content in both groups.

[a]Indicates that the mean of the oxidized LDL is significantly ($P < 0.05$) different from the mean of the native LDL in the same subject group.

[b]Indicates that the mean of the patient group is significantly ($P < 0.05$) different from the corresponding normal group.

The effect of LDL containing lysoPC with various acyl chain lengths on endothelium-dependent relaxation was investigated. Native LDL (0.06 μmol lysoPC/mg LDL protein) was incubated with 0.5 mM lauroyl- or palmitoyl-lysoPC for 24 h, and the loaded LDL was then dialyzed for 24 h with three changes of buffer to remove any free lysoPC in the solution. The amount of lysoPC in the LDL sample after treatment was found to increase from 0.06 μmol/mg LDL protein to 1.56 μmol/mg LDL protein. The amounts of PC and sphingomyelin in the LDL sample were not altered by this treatment. The LDL containing the lauroyl-lysoPC did not produce any additional impairment of endothelial relaxation when compared with the native LDL (control, **Fig. 3**). The LDL containing the palmitoyl-lysoPC, how-

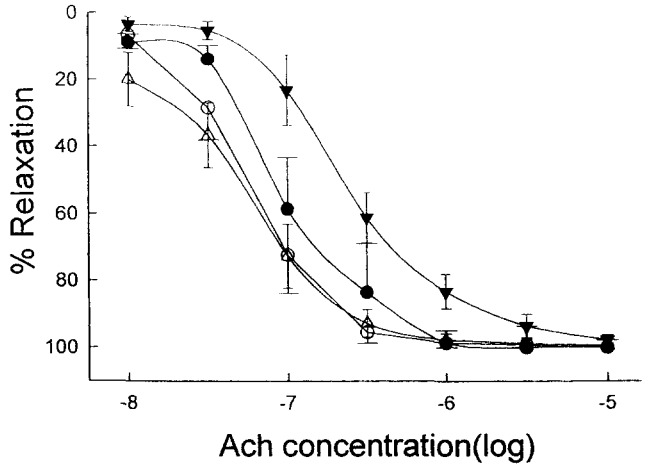

Fig. 2. Direct effect of the medium- (C_{12-14}) and long-chain (C_{16-18}) lysoPC on the impairment of endothelium-dependent relaxation of the rat aortic ring. The impairment of endothelial dependent relaxation of the rat aortic ring was studied in the absence (control, ○) or presence of 10 μM of 1-lauroyl-*sn*-glycero-3-phosphocholine ($C_{12:0}$-lysoPC) (△), 1-myristoyl-*sn*-glycero-3-phosphocholine ($C_{14:0}$-lysoPC, ●) or 1-palmitoyl-*sn*-glycero-3-phosphocholine ($C_{16:0}$-lysoPC, ▼). Each point represents the mean of at least three separate experiments, each done in duplicate. The vertical bar represents the SEM.

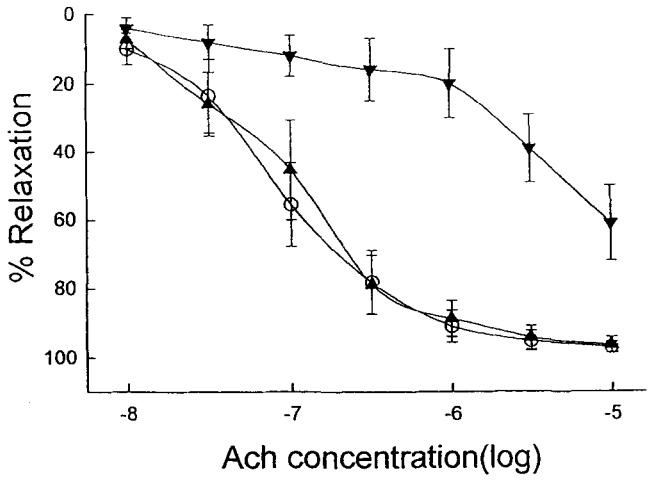

Fig. 3. Effect of lysophosphatidylcholine-treated native low density lipoprotein on the impairment of endothelium-dependent relaxation of the rat aortic ring. The aortic ring preparation was exposed to LDL loaded with 1-lauroyl-*sn*-glycero-3-phosphocholine ($C_{12:0}$-lysoPC, ▲) or 1-palmitoyl-*sn*-glycero-3-phosphocoline ($C_{16:0}$-lysoPC, ▼) for 1 h. Native LDL (without lysoPC incubation, ○) was used as control. Each point represents the mean of at least three separate experiments, each done in duplicate. The vertical bar represents the SEM.

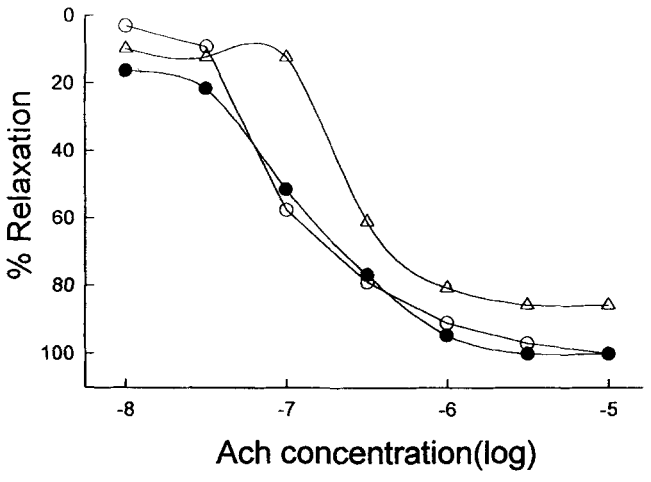

Fig. 4. Effect of increased long-chain lysoPC content in oxidized LDL from normal subjects on endothelium-dependent relaxation of the rat aortic ring. The aortic ring preparation was exposed to normal oxidized LDL that had been equilibrated with 1-palmitoyl-*sn*-glycero-3-phosphocholine ($C_{16:0}$-lysoPC) to increase its long-chain lyso-PC content from 0.342 (untreated, ○) to 0.367 (●) and 0.405 (△) μmol/mg LDL protein. The result depicted is typical of two similar experiments on oxidized LDL samples from two normal subjects.

ever, produced a significantly greater impairment of the endothelium-dependent relaxation than did the native LDL or LDL loaded with the medium-chain lysoPC.

It is clear that the impairment of endothelium-dependent relaxation of the aortic ring is dependent on the chain-length of the lysoPC acyl group. The difference, however, between long-chain lysoPC content of oxidized LDL from normal and hyperlipidemic patients, although significant, was quantitatively small. Hence, the functional significance of the change in lysoPC composition between the two groups was investigated. Our approach was to obtain oxidized LDL from normal individuals, and to modify its lysoPC content and composition so that it would mimic the oxidized LDL from hyperlipidemic patients. The abilities of the unmodified and modified oxidized LDL to impair the endothelium-dependent relaxation were then examined. Typically, 3.5 ml of the oxidized LDL (1 mg protein/ml) from normal subjects, containing 0.342 μmol of lysoPC/mg protein, was incubated with 0.238 μmol palmitoyl-lysoPC for 24 h at room temperature. After incubation, the mixture was dialyzed to remove the free lysoPC. The modified LDL had lysoPC content of 0.405 μmol/mg protein, which indicated that over 90% of the exogenous lysoPC was bound to the oxidized LDL. The content and composition of lysoPC in the oxidized and modified LDL from normal subjects (0.405 μmol/mg protein, 83% long chain acyl group) approaches that found in hyperlipidemic patients (0.40 μmol/mg protein, 94% long chain acyl group). The effect of the modified oxidized LDL on endothelium-dependent relaxation was examined and compared with that obtained from unmodified samples. The results (**Fig. 4**) show a considerable difference in the impairment of endothelium-dependent relaxation between the modified and unmodified oxidized LDL. As depicted in Fig. 4, the impairment of endothelium-dependent relaxation appears to be potentiated upon increasing lysoPC concentration between 0.37 and 0.40 μmol/mg protein which is similar to that seen between normal and hyperlipidemic patients.

DISCUSSION

The present study was designed to investigate the effect of oxidatively modified LDL from normal and hyperlipidemic patients on endothelium-dependent relaxation. As was expected, high levels of total cholesterol, LDL cholesterol, and triglyceride were detected in the plasma of hyperlipidemic patients. Unmodified LDL from hyperlipidemic patients did not impair the endothelium-dependent relaxation. Of particular interest, however, was the greater impairment of the endothelium-dependent relaxation by the oxidatively modified LDL from hyperlipidemic patients. These results are functionally consistent with previous findings that LDL levels in hyperlipidemic patients are associated with loss of endothelial function and constriction of blood vessels (5) and that the latter

are produced by oxidatively modified LDL (20–22, 27, 28, 30).

Analysis of the lipid content in the oxidatively modified LDL from both subject groups revealed that the level of lysoPC was greatly elevated, with a corresponding decrease in the level of PC. Our hypothesis was that a higher level of lysoPC would be present in the oxidized LDL of the hyperlipidemic group. Surprisingly, only a small increase (17%) in lysoPC was detected in the oxidized LDL samples from hyperlipidemic subjects. This finding was unexpected as *a*) the oxidatively modified LDL from hyperlipidemic patients has been shown to produce a stronger impairment of the endothelium-dependent relaxation, and *b*) an elevated level of lysoPC has been postulated to be a factor responsible for that impairment (20, 22). At present, the biochemical event leading to the formation of lysoPC from PC during oxidative modification remains undefined. The hydrolysis of PC by the action of phospholipase A_2 during oxidative modification has been suggested (37).

It should be noted that lysoPC is not a single entity, but is composed of molecular species with different acyl groups (38). The physical and biological properties of lysoPC are dependent on the chain length of the constituent acyl groups (39). Molecular species with long acyl chains are more hydrophobic (39) and any increase in their proportion may alter the biological properties of the LDL. In this study, we provide evidence for the first time that the oxidative modification of LDL produces changes in the acyl composition of lysoPC. These changes are characterized by an elevated long-chain acyl content which is further increased in the LDL from hyperlipidemic subjects.

This study is also the first to demonstrate that the ability of lysoPC to impair endothelium-dependent relaxation is a function of the chain length of the acyl group. In native LDL, samples from hyperlipidemic patients show a significantly elevated proportion of long chain acyl groups in lysoPC. The quantitatively small amount of lysoPC in these samples, however, is consistent with the minimal effect they have on endothelium-dependent relaxation. Upon oxidative modification, however, the dramatic increase of lysoPC content, together with a higher proportion of long-chain ($C_{\geq 16}$) acyl content, should provide the oxidized LDL with an enhanced effectiveness in impairing endothelium-dependent relaxation. The differential effect of the molecular species of lysoPC on the impairment of the endothelium-dependent relaxation has been demonstrated with exposure of the aortic ring preparation to *1*) free lysoPC, *2*) native LDL loaded with lysoPC, and *3*) oxidized normal LDL whose long-chain lysoPC content was experimentally elevated to mimic the level found in the oxidized LDL of hyperlipidemic subjects. The observation of a sharp increase in the sensitivity of endothelium-dependent relaxation at lysoPC concentrations between those seen in normal and hyperlipidemic individuals is suggestive of a functional threshold for inhibition of the endothelial control within that range of lysoPC concentrations. The results obtained in this study provide a rational explanation for the greater impairment of endothelium-dependent relaxation by the oxidized LDL of hyperlipidemic patients. In view of the small increase (17%) in lysoPC content found in the oxidized LDL of hyperlipidemic patients, it is reasonable to conclude that the higher level of the long-chain moiety found in these patients is responsible for its enhanced ability to impair endothelium-dependent relaxation of the vascular preparation. We propose that the high level of LDL found in the plasma of hyperlipidemic patients, coupled with the enhanced ability to generate long-chain species of lysoPC during oxidative modification, are important factors that contribute to the development of atherosclerosis in these patients.

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