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Increased lysophosphatidylcholine and non-esterified fatty acid content in LDL induces chemokine release in endothelial cells. Relationship with electronegative LDL.

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Abstract

Electronegative low-density lipoprotein (LDL(−)) is a plasma-circulating LDL subfraction with proinflammatory properties that induces the production of chemokines in cultured endothelial cells. However, the specific mechanism of LDL(−)-mediated chemokine release is presently unknown. A characteristic feature of LDL(−) is an increased content of lysophosphatidylcholine (LPC) and non-esterified fatty acids (NEFA). The effect of increasing amounts of LPC and NEFA associated with LDL on the release of chemokines by endothelial cells was studied. Total LDL was subfractionated by anion-exchange chromatography in electropositive (LDL(+)) and LDL(−). LDL(−) contained two-fold more LPC and NEFA than LDL(+) and induced two- to four-fold more ($p < 0.05$) interleukin-8 (IL-8, 11.5 ± 8.2 ng/10^5 cells) and monocyte chemotactic protein-1 (MCP-1, 10.8 ± 3.8 ng/10^5 cells) release by human umbilical vein endothelial cells (HUVEC) than LDL(+) (IL-8: 3.4 ± 1.5 ng/10^5 cells, MCP-1: 5.8 ± 2.9 ng/10^5 cells). The content of LPC and NEFA in LDL(+) was increased by enzymatic treatment with secretory phospholipase A_2 ($sPLA_2$) at 5 ng/mL or 20 ng/mL or by incubation with NEFA at 2 mmol/L. Modification of LDL(+) by both methods did not result in oxidative modification as demonstrated by the lack of change in antioxidants, conjugated dienes and malondialdehyde content. $sPLA_2$ treatment resulted in an increase in LPC and NEFA in LDL(+) which enhanced its ability to release IL-8 and MCP-1 by HUVEC in a concentration-dependent

manner (sPLA$_2$(5)-LDL; IL-8: 7.1 ± 3.8 ng/10^5 cells, MCP-1: 8.0 ± 5.1 ng/10^5 cells; sPLA$_2$(20)-LDL; IL-8: 20.8 11.2 ng/10^5 cells, MCP-1: 15.0 7.5 ng/10^5 cells). NEFA loading of LDL(+) also favored the release of IL-8 and MCP-1 (IL-8: 7.8 ± 6.1 ng/10^5 cells, MCP-1: 8.4 ± 2.7 ng/10^5 cells, $p < 0.05$ versus LDL(+)). These effects were observed when modified LDL(+) reacheda content of LPC and/or NEFA similar that of LDL(−). These data indicate that non-oxidized polar lipids associated with LDL promotean inflammatory response in endothelial cells and suggest that increased NEFA and LPC could be involved in the inflammatory activity of LDL(−).

Keywords: Electronegative LDL; Chemokines; Lysophosphatidylcholine; Non-esterified fatty acids; Inflammation

1. Introduction

Qualitative modifications in low-density lipoprotein (LDL) are a key factor in the initiation and development of atherosclerosis. LDL trapped in the arterial intima is modified by several mechanisms such as oxidation, aggregation, proteoglycan–LDL complex formation or degradation by lipolytic enzymes, all of which are frequently inter-related [1]. These modified LDLs induce the uncontrolled accumulation of lipids in the arterial wall which produces the formation of foam cells and triggers the inflammatory response characteristic of atherogenesis, which includes endothelial dysfunction, leucocyte recruitment and differentiation and smooth muscle cell proliferation [2]. In addition to the occurrence ofmodified LDL in the arterial intima, several modified LDL forms have also been detected in blood. Among them, an electronegatively charged LDL subfraction (LDL(−)) has been described in plasma in a variable proportion ranging from1 to 10% in healthy normolipemic subjects [3–6]. LDL(−) proportion is increased in diseases with high cardiovascular risk such as familial hypercholesterolemia [7], hypertriglyceridemia [8], diabetes mellitus types 1 and 2 [9–11] or kidney failure [12]. In vitro studies showed that LDL(−) is cytotoxic to cultured endothelial cells [13,14] and inducesthe release of chemokines such as interleukin-8 (IL-8) and monocyte chemotactic protein 1 (MCP-1) involved in leucocyte recruitment [15,16]. However, the mechanisms by which LDL(−) is generated are not fully understood. Some authorssuggested that LDL(−) could be of oxidative origin; since anincreased content of lipid oxidation products was observed [3,13,17,18]. In contrast, other authors found no evidence of oxidative modification in LDL(−) and attributed its increased negative charge to differences in density, size and lipid and apoprotein composition [4,5,8,14–16]. Further evidence thatLDL(−) production could be unrelated to oxidative modification is the observation that LDL(−) is increased in diseases such as familial hypercholesterolemia [7] and type 1 diabetes mellitus [9,10] in which total LDL susceptibility to oxidationis

similar or even lower than that observed in healthy subjects. Among differences observed in lipid and apoprotein composition between LDL(−) and native LDL, our group reportedthat LDL(−) present increased the content of non-esterifiedfatty acids (NEFA) compared to the non-electronegative LDL fraction (LDL(+)) [15,16], and other authors also observed increased lysophosphatidylcholine (LPC) content in LDL(−) [17]. Since both NEFA and LPC are products of phospho- lipase activity, we hypothesized that these enzymes could be related to the generation and inflammatory activity of LDL(-). In the current study, native human LDL was modified in vitro by secretory phospholipase A_2 ($sPLA_2$) from honey bee venom and the role of increased NEFA or LPC content in LDL on the release of IL-8 and MCP-1 was studied. Modified LDL(+) with a content of NEFA or LPC similar to that observed in LDL(−) shared similar proinflammatorycharacteristics with LDL(−), suggesting a major role of these polar lipids in the inflammatory action of LDL(−).

2. Methods

1.1. Isolation of LDL(+) and LDL(−)

Plasma samples from healthy normolipemic subjects (total cholesterol < 5.2 mmol/L, triglyceride < 1 mmol/L) were obtained in EDTA-containing Vacutainer tubes. Total LDL (1.020–1.050 g/mL) was isolated by sequential flotation ultracentrifugation [19] at 4 °C in presence of 1 mmol/L EDTA. Two fractions of LDL differing in their electric charge were separated by preparative anion-exchange chromatography in an ÄKTA-FPLC system with a HiLoad 26/10 Q Sepharose high-performance column (Amersham Pharmacia) using a multistep NaCl gradient, as described [15] with some modifications. LDL was dialyzed against buffer A (Tris 10 mmol/L, EDTA 1 mmol/L, pH 7.4) and 20–30 mg of apoB was injectedinto the column at a flow rate of 6 mL/min. The gradient was as follows: 1 column volume 0% buffer B (0–53 mL, bufferB was Tris 10 mmol/L, NaCl 1 mol/L, EDTA 1 mmol/L, pH 7.4), 1 column volume 0–10% buffer B (53–106 mL), 2 column volumes 24% buffer B (106–212 mL), 2 column volumes 60% buffer B (212–318 mL), 2 column volumes 100% buffer B (318–424 mL); the column was then re- equilibrated with 3 column volumes of buffer A. LDL(+) eluted at 0.24 mol/L NaCl and LDL(−) eluted at 0.6 mol/L NaCl. Both fractions were concentrated by ultracentrifugation, and lipid and apoprotein composition was determined by commercially available methods in a Hitachi 911 autoanalyzer, as described [15]. Results were expressed as % of LDL mass or as mol/mol apoB. Agarose electrophoresis (Midigel, BioMidi) was routinely performed in all preparations to con- firm increased electronegativity of LDL(−). LDL size was determined by non-denaturing polyacrylamide gradient gel electrophoresis, as described [8].

1.2. *Preparation of modified LDLs*

NEFA-enriched LDL (NEFA-LDL) was obtained by incubation of LDL(+) (0.5 g apoB/L) with a mixture of NEFA (palmitic/oleic/linoleic acids in proportion 35:20:45; final concentration 2 mmol/L) for 4 h at 37 °C, as described [20], except that lipoprotein-deficient serum (LPDS) was substituted by 45 g/L of fatty acid-free albumin (FAF-albumin, ref.# A6003 Sigma). $sPLA_2$-treated LDL ($sPLA_2$-LDL) was obtained by incubation of total LDL (1 g apoB/L) with increasing concentrations of purified $sPLA_2$ (from honey bee venom, ref. # P9279, Sigma) (5 and 20 µg/L) for 2 h at 37 °C, as de- scribed [21]. In all cases LDL(+) was modified in the presence of 1 mmol/L EDTA, 2 µmol/L butylated hydroxytoluene (BHT) and 45 g/L FAF-albumin. Control LDL(+) was incubated in the same conditions as NEFA-LDL or $sPLA_2$-LDL.After modification, all LDLs were concentrated by flotationultracentrifugation for 12 h at 100,000 x *g* and 4 °C (density 1.050 g/mL) to eliminate albumin and/or $sPLA_2$ and filtered through 0.22 µm. The degree of modification was evaluated by agarose electrophoresis and by measuring NEFA and LPC bound to LDL. The possibility of oxidative modification of LDL(+) during incubation was assessed by measuring conjugated dienes at 234 nm [22], malondialdehyde [23] and antioxidant content [6]. In addition, LDL susceptibility to oxidation was also determined by monitoring conjugated dienes formation, as described [20,22].

1.3. Phospholipid quantification

Phospholipids in LDL were quantified by normal-phase HPLC as described [24], with some modifications. Dipalmitoyl-glycero-phosphodymethyl ethanolamine (DGPE, ref. # P0399 Sigma) (final concentration 2 g/L) was used as internal standard. Briefly, 50 µL of DGPE at 20 g/L in ethanol was added to 450 µL of LDL (0.5 g apoB/L) and lipids were extracted by the Bligh and Dyer method [25]. Pellet was resuspended with 200 µL of hexane:isopropanol:water (vol:vol:vol, 6/8/1) and 20 µL of this solution were injected into a normal-phase column (Hibor Lichrosorb Si60, 5 µm, Merck) at a flow rate of 0.6 mL/min. Mobile phase was acetonitrile:methanol:ammonium sulphate 5 mmol/L (vol:vol:vol, 56/23/6). Peaks were detected at 206 nm using a Photo Diode Array detector (Model 168, Beckman). Phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphyngomyelin (SM) and lysophosphatidylcholine (LPC) peaks were resolved by this method.

1.4. Endothelial cell culture

Human umbilical vein endothelial cells (HUVEC) were isolated by collagenase digestion and cultured as described [16]. Cells in confluent state (one to two passages) were seeded in six-well plates at 10,000 cells/cm^2. When cells reached confluence, LDLs were added for a subsequent 24 hat 150 mg apoB/L in 199 medium containing 1% fetal calf serum (FCS) without heparin and endothelial cell growth supplement (ECGS).

LDLs were previously dialyzed against 199 medium and filtered through 0.22 µm. Interleukin-1β (IL-1β) (20 µg/L) was used as a positive control for chemokine production. Cell viability was evaluated by optic microscopy. No cell detachment or gaps were observed after incubation with LDLs. We previously reported that under these cultureconditions, neither LDL(+) nor LDL(−) induced cell death,measured by MTT or propidium iodide tests [15,16]. After 24-h incubation of HUVEC with LDLs or IL-1β, supernatant aliquots were centrifuged to eliminate debris and frozen at 40 °C for IL-8 and MCP-1 measurements. IL-8 (Endogen)and MCP-1 (Pharmingen) released in the media were quantified by ELISA. Results were expressed as ng/10^5 cells.

1.5. Statistical analysis

Results are expressed as mean ± S.D. Differences betweengroups were tested with Wilcoxon's *t*-test (for paired data) and Mann–Whitney *U*-test (for unpaired data). A value of $p < 0.05$ was considered significant.

2. Results

The composition of LDL subfractions is shown in Table 1; in agreement with previous results [15,16], LDL(−) presented a lower proportion of apoB and an increased proportion of triglyceride than LDL(+) as well as a two-fold in- crease in NEFA in LDL(−). Analysis of major phospholipids revealed that LPC was increased two-fold in LDL(−) com-pared to LDL(+) (54.8 ± 23.3 versus 28.5 ± 16.5 mol/mol apoB, respectively, $p < 0.05$, Table 1) with no differences inthe rest of phospholipids analyzed. A representative chromatogram of LDL(+) and LDL(−) phospholipids is shown in Fig. 1.

Incubation of LDL(+) with 2 mmol/L NEFA resulted in an eight-fold increase in these compounds remaining associated with LDL (Table 2). However, the remaining lipid components, electric charge and particle size were unchanged after NEFA-loading (data not shown). Regarding $sPLA_2$ treatment, a progressive decrease in total phospholipids and PC in LDL(+) was observed with a simultaneous increase in both LPC and NEFA (Table 2). PE also decreased after $sPLA_2$ treatment whereas SM content was not modified (data not shown). However, the decrease in PC was not accompaniedby an equimolar increase in LPC and NEFA as theoreticallyexpected. This was due to the presence of albumin in the incubation mixture which causes a partition of the most po-lar lipids, in this case LPC and NEFA, from LDL to albumin. $sPLA_2$ treatment resulted in a progressive increase in LDL electric charge determined by agarose gel electrophoresis and decrease in LDL size determined by gradient gel electrophoresis (data not shown). Neither $sPLA_2$ treatment nor NEFA loading resulted in the increase in conjugated dienes or MDA content in LDL. Antioxidants, including α- tocopherol, lycopene, α-carotene and β-carotene, were alsounchanged after

lipoprotein modification (Table 2). Further confirmation of the lack of oxidation after modification of LDL is the finding that $sPLA_2$-treatment or NEFA-loading promoted an increase in the resistance to oxidation of LDL (Fig. 2). The increase in NEFA and LPC in LDL(+) resulted in an induction of chemokine release from HUVEC. $sPLA_2$-LDL induced chemokine release from HUVEC in a concentration-dependent manner (Fig. 3). LDL(+)-treatment with 20 μg/L of $sPLA_2$ increased IL-8 and MCP-1 release more than that observed with LDL(−). Regarding the action of NEFA-enriched LDL, stimulation of IL-8 and MCP-1 release at a level similar to that displayed by LDL-PLA_2 at5 μg/L was observed (Fig. 3). This observation indicates thatNEFA alone, even at high amounts associated with LDL, a weaker stimulator of chemokine production than NEFA combined with LPC.

3. Discussion

Recent findings suggest that LDL(−) could play a relevantrole in atherogenesis through the production of proinflammatory molecules by endothelium since these lipoproteins induce the release of IL-8 and MCP-1 in cultured HUVEC [15,16]. These chemokines are involved in the recruitment of leucocytes to the lesion areas in the vessel wall, and theirplasma concentrations are elevated in FH subjects [26,27]. There is evidence relating LDL(−) to high chemokine levelsin plasma. FH patients share a high proportion of LDL(−) and high plasma levels of MCP-1 and IL-8 and statin therapy decreases both LDL(−) [7] and chemokine plasma concentration [27]. Interestingly, this statin-induced decrease inLDL(−) and chemokine is delayed (several months) compared with the rapid effect on cholesterol plasma levels (one month or less). However, the mechanisms whereby LDL(−) induce the release of chemokines remain unknown. Controversy has been raised concerning the presence [3,13,17,18] or absence [4,5,14–16] of oxidized lipids in LDL(−). It hasbeen reported recently that the most electronegative fractionof LDL from FH subjects, but not from normolipemic subjects, presents slightly increased oxidation markers [28,29].However, we observed that LDL(−) from normolipemic subjects has similar proinflammatory activity to LDL(−) from FH subjects [16,30]. For this reason we explored the possibility that non-oxidized lipids could mediate the inflammatory properties of LDL(−). Results obtained in the current work support a relevant role of polar, non-oxidized lipids in the proinflammatory activity of LDL(−).

Our results confirm the increased NEFA and LPC con- tent in LDL(−) compared to LDL(+), in agreement with previous reports [15–17]. The amount of LPC associated with LDL measured in the current study (LDL(+) 2.4%; LDL(−) 4.7%) is in good agreement with those reported by Sevanian et al. (LDL(+) 2.0%; LDL(−) 3.5%). Both NEFA and LPC are inflammation mediators known to mediate the production of cytokines, growth factors and other inflammatory molecules through the activation of transcription factors related to

atherogenesis, such as nuclear factor κB (NF-κB) or activator protein 1 (AP-1) [31,32]. Data obtained with sPLA2-modified LDL suggest a relevant role for NEFA and/or LPC in the LDL(−)-mediated relase of chemokines in cultured HUVEC. Increased NEFA and LPC induced by $sPLA_2$-treatment conferred inflammatory activity on LDL(+) similar to that observed in LDL(−), with the amount of IL-8 and MCP-1 released being dependent on the amount of NEFA and/or LPC generated by sPLA2. This effect of sPLA2-modified LDL was observed in absence of increased oxidized products or consumption of antioxidants which outlines a role of polar, non-oxidized lipids in the proinflammatory action of lipoproteins. Further indication that the induction of chemokine release is unrelated to oxidized lipids is the finding that both sPLA2-modification and NEFA-loading yielded LDL particles more resistant to oxidation, in accordance with the increased resistance to oxidation of LDL(−) from normolipemic and hypercholesterolemic subjects [16]. Our results also concur with those recently reported by Sonoki et al. who observed increased MCP-1 expression induced by PLA_2-treated LDL [33], although they found induction of MCP-1 mRNA synthesis with a 10-fold increase in LPC con- tent in LDL. Our data indicate that a three-fold increase in LPC is sufficient to increase two-fold the release of MCP-1 and IL-8 protein.

To distinguish the separate roles of NEFA and LPC, LDL(+) was loaded with a mixture of NEFA. NEFA-LDL had a mild effect on the release of MCP-1 and IL-8, since although its NEFA content was five-fold higher than that observed in LDL(−) the chemokine release was intermediate between LDL(+) and LDL(−). This observation suggests that LPC plays a major role in the chemokine release induced by LDL(−). Nevertheless, it is noteworthy that increased NEFA content in LDL induces 'per se' the release of IL-8 and MCP-1. This concurs with a recent paper by Suriyaphol et al. reporting that enzymatically modified LDL (E-LDL) induced the expression of IL-8 in endothelial cells [34], with this action of E-LDL being mainly due to its increased content in NEFA.

The origin of increased NEFA and LPC in LDL(−) is unknown. Increased NEFA content could originate from the enzymatic hydrolysis of triglycerides, esterified cholesterol or phospholipids in LDL; in this context, the possible role of lipoprotein lipase, hepatic lipase, cholesteryl esterase or several phospholipases in the enrichment of NEFA in LDL(−) should be studied in detail in further works. Nevertheless, NEFA associated with LDL could also increase in situations in which the buffering ability of albumin to bind NEFA is overcome [35]. We previously reported that the increase in NEFA in plasma due to energy requirements after intense aerobic exercise results in parallel increases in NEFA associated with LDL and in enhanced LDL(−) proportion [20]. Thus, a decrease in albumin or an increase in NEFA concentrations in plasma could lead to higher amounts of NEFA associated with LDL and could therefore favor the generation of LDL(−).

Unlike NEFA, the increase in LPC in LDL(−) is a consequence of phospholipase A_2 activities. We used secretory $sPLA_2$, an enzyme whose expression is upregulated by inflammatory mediators [36] and whose concentration is high in atherosclerotic lesions [37]. In the arterial wall $sPLA_2$ can hydrolyze lipoproteins and induce their aggregation and fusion which has been postulated as a key factor to enhance their retention in arterial intima [38,39]. Hence, LDL(−) could be produced in the vascular wall and represent particles originating from a reverse traffic from arterial intima, as hypothesized by Avogaro and co-workers [40]. On the other hand, circulating $sPLA_2$, which is mainly produced in plasma by monocytes, could modify plasma LDL, thereby contributing to the generation of atherogenic lipoproteins [38,39].

Nevertheless, probably the most important phospholipase A_2 activity in plasma comes from platelet-activating factor acetylhydrolase (PAF-AH). This enzyme is carried by lipoproteins and has PAF and other PAF-like phospholipids as substrates [41], and generates LPC and fractionated and/or oxidized NEFA that could induce inflammation [42]. The possibility that PAF-AH could play a determinant role in the generation of LDL(−) and in their inflammatory action is sup-ported by the recent finding that PAF-AH preferently binds to LDL(−) [43]. However, since PAF-AH only hydrolyzes oxidized phospholipids, this hypothesis would imply that, at the very least, a low degree of oxidative modification should occur in LDL(−). It is tempting to speculate that PAF-AH could bind to LDL in response to incipient oxidation with the objective of inactivating PAF-like phospholipids; as a result, LPC and fractionated NEFA increase in LDL yielding LDL(−). It is noteworthy that our method detects short-chain NEFA with more than six carbon atoms, which could come from the degradation of the *n* 2 fatty acyl of phospholipids. Moreover, the fact that most of these short-chain NEFA tend to leave from LDL could explain the lack of oxidative modifications that we and others found in LDL(−). Nevertheless, owing to the intrinsic unstable nature of oxidation products, the presence of small amounts of oxidized lipids in LDL(−) cannot be ruled out.

In summary, our findings indicate that increasing NEFA and LPC in LDL(+) at similar levels to those displayed by LDL(−) render these particles proinflammatory. Nevertheless, the mechanisms involved in the generation of LDL(−) enriched in LPC and NEFA remain poorly understood and further studies are required.

Acknowledgments

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Table 1

Composition of LDL subfractions

	LDL(+)	LDL(−)
apoB[a]	25.7 ± 1.2	23.9 ± 1.9*
Total cholesterol[a]	39.0 ± 1.3	38.6 ± 1.2
Free cholesterol[a]	11.3 ± 0.4	12.1 ± 0.9
Triglyceride[a]	7.5 ± 0.9	9.0 ± 1.3*
Total phospholipid[a]	27.8 ± 2.1	28.6 ± 1.7
NEFA[b]	12.9 ± 1.3	30.7 ± 4.3*
Phosphatidylcholine[b]	735.2 ± 148.4 (63.4)[c]	786.4 ± 175.6 (64.8)
Sphingomyelin[b]	262.6 ± 65.4 (22.7)	233.0 ± 53.0 (19.2)
Phosphatidylethanolamine[b]	133.2 ± 32.5 (11.5)	136.1 ± 38.3 (11.2)
Lysophosphatydylcholine[b]	27.99 ± 15.4 (2.4)	57.8 ± 22.9* (4.7)

[a] Expressed as mean ± S.D. in percent of total LDL mass.

[b] Expressed as mean ± S.D. in mol/mol apoB.

[c] In parentheses indicate the percent of total phospholipids.

* $p < 0.05$ vs. LDL(+) ($n = 9$).

Table 2

Effect of incubation of LDL(+) with NEFA and PLA_2 on the content of PC, LPC, NEFA, oxidation products and antioxidants

	LDL(+)	LDL-PLA_2 (5 μg/L)	LDL-PLA_2 (20 μg/L)	LDL-NEFA (2 mmol/L)
NEFA[a]	12.9 ± 1.3	41.3 ± 9.8*	61.2 ± 16.2*	100.6 ± 69.8*
Total phospholipids[b]	28.6 ± 1.7	22.7 ± 2.1*	19.3 ± 1.5*	28.9 ± 1.0
PC[a]	735.2 ± 148.4	403.6 ± 67.2*	195.2 ± 68.0*	ND
LPC[a]	27.9 ± 15.4	92.6 ± 29.4*	155.5 ± 76.1*	ND
MDA[a]	0.44 ± 0.16	0.42 ± 0.07	0.37 ± 0.06	0.40 ± 0.25
Conjugated dienes[c]	0.350 ± 0.055	0.360 ± 0.010	0.380 ± 0.010	0.300 ± 0.040
α-Tocopherol[c]	6.79 ± 1.13	6.76 ± 1.25	6.44 ± 1.23	7.24 ± 1.22
α-Carotene[a]	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.05 ± 0.02
β-Carotene[a]	0.17 ± 0.05	0.17 ± 0.05	0.16 ± 0.03	0.15 ± 0.10
Lycopene[a]	0.28 ± 0.06	0.21 ± 0.11	0.18 ± 0.08	0.27 ± 0.11

[a] Expressed as mol/mol apoB.

[b] Expressed as percentage of total LDL mass.

[c] Expressed as absorbance units. Results are expressed as mean ± S.D. (n = 6), except LDL(+) (n = 9). ND, not determined.

* $p < 0.05$ vs. LDL(+).

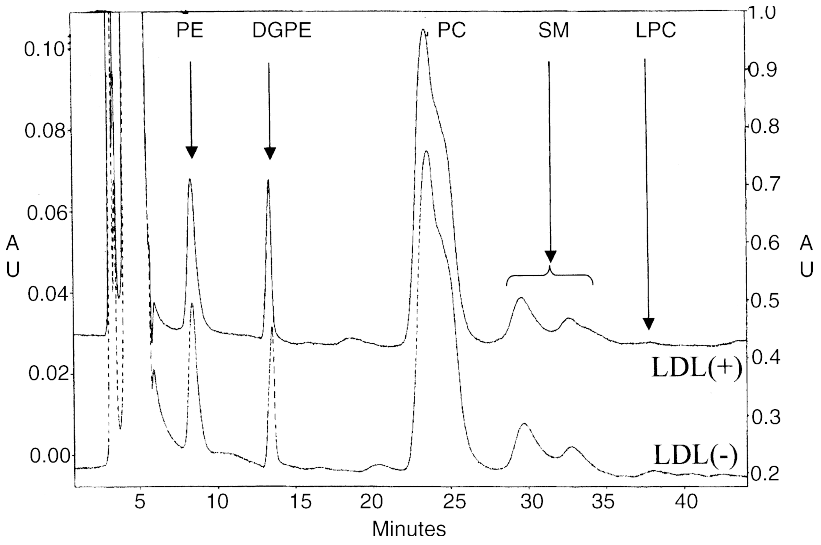

Fig. 1. Representative chromatogram of major phospholipids from LDL(+) (upper chromatogram) and LDL(–) (lower chromatogram). Total lipids were extracted according to Bligh and Dyer and high-performance liquid chromatography (HPLC) was developed in a normal-phase column, as described in Section 2. PE, phosphatidylethanolamine; DGPE, dipalmitoyl-glycero-phosphodymethyl ethanolamine; PC, phosphatidylcholine; SM, sphyngomyelin; LPC, lysophosphatidylcholine.

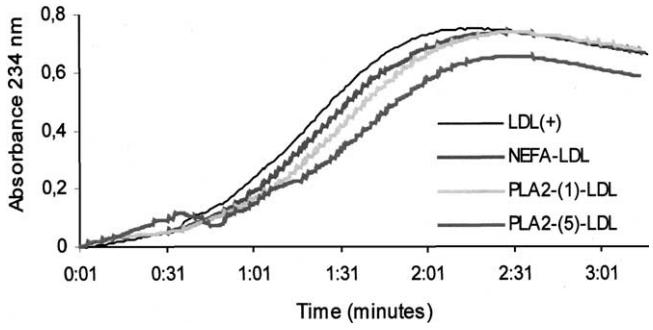

Fig. 2. LDL susceptibility to oxidation. Representative conjugated diene formation kinetics of $sPLA_2$-LDL and NEFA-LDL. LDLs (50 mg apoB/L dialyzed in PBS) were incubated with 2.5 µmol/L $CuSO_4$ at 30 ˚C and the formation of conjugated dienes was monitored at 234 nm.

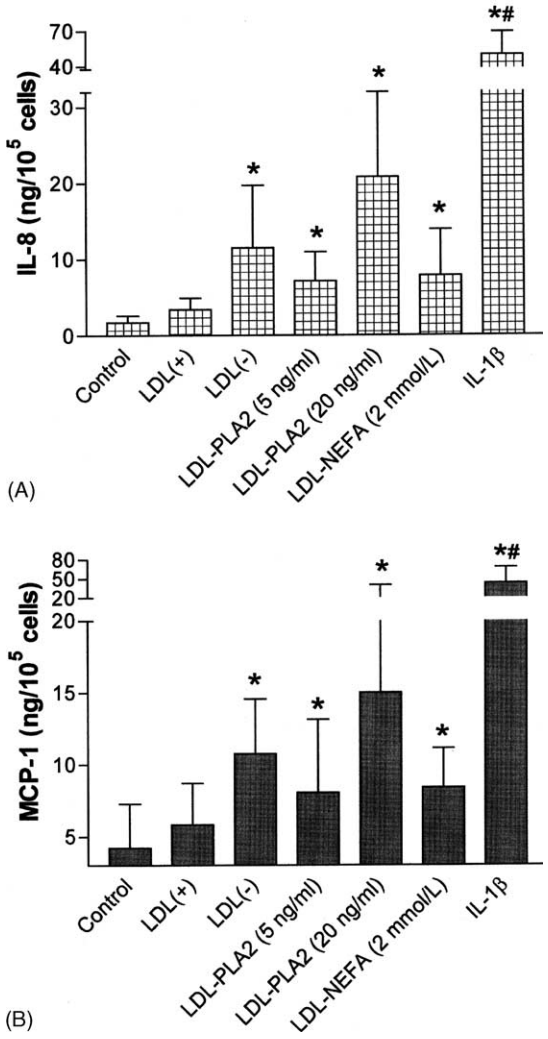

Fig. 3. Induction of IL-8 (A) and MCP-1 (B) release from HUVEC induced by different LDLs. Cells were grown as indicated in methods and incubated with 150 μg/mL of each LDL or 20 μg/L IL-1β for 24 h. Chemokines were measured from supernatant by commercial ELISA. * $p < 0.05$ vs. LDL(+); # $p < 0.05$ vs. LDL(−).