# Electronegative LDLs from familial hypercholesterolemic patients are physicochemically heterogeneous but uniformly proapoptotic

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**Abstract    A highly electronegative fraction of human plasma LDLs, designated L5, has distinctive biological activity that includes induction of apoptosis in bovine aortic endothelial cells (BAECs). This study was performed to identify a relationship between LDL density, electronegativity, and biological activity, namely, the induction of apoptosis in BAECs. Plasma LDLs from normolipidemic subjects and homozygotic familial hypercholesterolemia subjects were separated into five subfractions, with increasing electronegativity from L1 to L5, and into seven subfractions according to increasing density, D1 to D7. L1 to L5 were also separated according to density, and D1 to D7 were separated according to charge. The density profiles of L1 to L5 were similar (maximum density = $1.030 \pm 0.002$ g/ml). Induction of apoptosis by all seven density subfractions was confined to the highly electronegative fraction, L5, and within each density subfraction the magnitude of apoptosis correlated with the L5 content.■ Electronegative LDL is heterogeneous with respect to density and composition, and induction of apoptosis is more strongly associated with LDL electronegativity than with LDL size or density.**—Chen, H-h., B. D. Hosken, M. Huang, J. W. Gaubatz, C. L. Myers, R. D. Macfarlane, H. J. Pownall, and C-y. Yang. **Electronegative LDLs from familial hypercholesterolemic patients are physicochemically heterogeneous but uniformly proapoptotic.** *J. Lipid Res.* 2007. **48:** 177–184.

**Supplementary key words**   low density lipoprotein • apoptosis • low density lipoprotein dense subfractions • atherosclerosis

Plasma levels of LDL-cholesterol are positively associated with the risk for coronary heart disease (CHD). Although some models suggest that oxidized LDLs are an important initiating factor for atherogenesis, their identity and structure in vivo are not known. Small, dense low density lipoprotein (sdLDL), another CHD risk factor,

may be mechanically linked to atherosclerosis (1–3) because of its susceptibility to oxidation (4, 5) and ready entry into the arterial wall (6). Other evidence suggests that LDL size is not independently linked to atherosclerosis or CHD. In some studies, large LDLs are associated with increased CHD (7–10), and LDL concentration and the ratio of LDL-cholesterol to HDL-cholesterol (11, 12) more reliably predict CHD. Therefore, the role of LDL size and density in atherogenicity remains unresolved.

LDL is distributed into multiple subclasses that can be isolated according to charge, density, and size, respectively, by ion-exchange chromatography, ultracentrifugal flotation, and size-exclusion chromatography (SEC). Electronegative low density lipoprotein (LDL⁻) (13, 14) isolated by ion-exchange methods is mechanically linked to atherosclerosis through its cytotoxicity to endothelial cells (ECs). LDL⁻ isolated from the plasma of patients with familial hypercholesterolemia and diabetes induces in vitro EC apoptosis and inflammatory responses through the induction of monocyte chemotactic protein-1 and interleukin-8 production (15, 16). Thus, the increased plasma LDL⁻ concentrations in FH and diabetic patients (17, 18) may increase CHD risk.

The molecular interactions associated with the biological effects of LDL⁻ are unknown. The altered conformation of apolipoprotein B-100 (apoB-100) in LDL⁻ is a factor in its reduced binding to the LDL receptor (19). Whereas one study showed that the densest LDL subfrac-

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Abbreviations: apoB-100, apolipoprotein B-100; BAEC, bovine aortic endothelial cell; CHD, coronary heart disease; EC, endothelial cell; EDGUC, equilibrium density gradient ultracentrifugation; FH, familial hypercholesterolemic; LDL⁻, electronegative low density lipoprotein; NaBiEDTA, sodium salt of a bismuth-EDTA complex; NBD $C_6$-ceramide, 6-{[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]hexanoyl}; NL, normolipidemic; REM, relative electrophoretic mobility; SEC, size-exclusion chromatography; sdLDL, small, dense low density lipoprotein.

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tions were richest in LDL⁻ (18%) (20), another reported more LDL⁻ in both sdLDL and buoyant LDL from hyperlipemic subjects (21). Reports on the distribution of LDL⁻ over the entire LDL density range have not appeared; this information could reveal the homogeneity of LDL⁻ and provide clues about its formation if it were associated with a narrow density range within the broader range of whole LDL.

Here, we report the density distribution of LDL⁻ isolated from hypercholesterolemic (FH) and normolipidemic (NL) subjects as assessed by equilibrium density gradient ultracentrifugation (EDGUC) (22) and an assay of biological effects, EC apoptosis.

## METHODS

### Materials

All reagents were purchased from Sigma (St. Louis, MO).

### Subjects

This study was conducted using LDL isolated from homozygotic FH and NL subjects. FH subjects (two females aged 33 and 41 years and one male aged 6 years when the project started) had total plasma cholesterol > 500 mg/dl. The diagnoses of homozygotic FH were made on the bases of DNA analyses of leukocytes and clinical features characteristic of the disease. The FH subjects were treated with cholesterol agents, such as lovastatin and atorvastatin, in addition to LDL apheresis. LDL apheresis was performed using a Liposorber LA-15 System (Kaneka America, New York, NY). Immediately after plasma was collected from blood by the plasma separator, EDTA, aprotinin, and sodium azide were added and processed by ultracentrifugation to obtain purified LDL. The three NL subjects, aged 40–55 years, had normal lipid values, including total cholesterol < 200 mg/dl, triglycerides < 160 mg/dl, and LDL cholesterol < 130 mg/dl. Lipoproteins were collected by plasmapheresis using an Autopheresis-C (Fenwal, Deerfield, IL). Aprotinin (0.056 U/ml plasma), sodium azide (0.06%, w/v), and EDTA (0.06%, w/v) were added to plasma immediately after collection. Consent forms, approved by the Institutional Review Board for Baylor College of Medicine and Affiliated Hospital, which describe the project procedure, potential risks, potential benefits, and subject rights, were obtained from plasma subjects before donation.

### LDL preparation

LDLs were isolated from plasma by sequential flotation between d = 1.019 and d = 1.063 g/ml (23), followed by flotation at d = 1.090 g/ml and dialysis against degassed 20 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, and 0.01% NaN₃ at 4°C with three buffer changes in 24 h.

### LDL charged fractionation

LDL was separated according to charge using a LCC-500 programmer controlling two P-500 pumps on a UnoQ12 column, an anion-exchange column (Bio-Rad, Hercules, CA) preequilibrated with buffer A (0.02 M Tris-HCl, pH 8.0, and 0.5 mM EDTA) at 4°C (17). LDL-protein in buffer A was loaded onto the UnoQ12 column and eluted with a multistep gradient of buffer B (1 M NaCl in buffer A): 0%, 10 min; 0–15%, 10 min; 15–20%, 30 min; isocratic 20%, 10 min; 20–40%, 25 min; 40–100%, 10 min; 100%, 15 min; 100–0%, 5 min; and 0%, 25 min. LDL fractions were pooled according to NaCl concentration into five subfrac-

tions, L1 through L5 (0.08–0.17, 0.17–0.18, 0.18–0.20, 0.20, and 0.20–0.38 M, respectively). The isolated fractions were concentrated, stored at 4°C, and analyzed within 2 weeks.

### LDL EDGUC fractionation

LDL (4 ml) isolated by flotation in KBr with d = 1.063 g/ml was transferred to centrifuge tubes (Beckman SW40 Ti). A step gradient of saline adjusted to d = 1.055, 1.050, 1.040, and 1.030 g/ml by the addition of KBr solution (24) was placed over the LDL, which was centrifuged at 35,000 rpm for 48 h at 4°C, after which the gradient was removed in 1 ml with a Pasteur pipette. The top 0.5 ml was discarded, and seven LDL subfractions with increasing density from D1 to D7 were collected; the bottom ∼1 ml was also collected but not included as LDL subfractions.

### Sodium salt of a bismuth-EDTA complex density profiling

LDL (10 μg of protein) was transferred to 1 ml centrifuge tubes and diluted with 20% sodium salt of a bismuth-EDTA complex (NaBiEDTA) to yield a 10% (w/v) solution of NaBiEDTA (ρ = 1.050 g/ml). NaBiEDTA solutions featuring a density gradient-forming solute are able to form self-generating gradients. Three microliters of 1 mg/ml 6-{[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]hexanoyl} (NBD C₆-ceramide) in DMSO was added and equilibrated with the LDL for 30 min, after which the samples were centrifuged for 6 h at 120,000 rpm and 5°C in a TLA 120.2 rotor (Beckman, Palo Alto, CA). The NaBiEDTA density gradient, based on the refractive index of 20 μl aliquots collected at various points in the gradient, was fitted to an exponential curve.

### Digital imaging and analysis

After centrifugation, tubes were placed in a custom fluorescence imaging station comprising a Plexiglas tube holder, a digital Optronics Microfire Camera (Goleta, CA), and a Dolan-Jenner (Lawrence, MA) MH-100 metal halide light source fitted with a liquid light guide (22). The tube holder, camera, and light source were mounted orthogonal to each other on an optical breadboard in a dark room. The camera lens and light guide were equipped with Schott Glass (Elmsford, NY) filters for NBD excitation (BG12) and emission (OG515) wavelengths. The image files were converted to an 1,800 × 1,200 matrix using Origin 7.0 (Microcal Software, Inc., Northampton, MA). The intensities of 20 columns corresponding to the center of the tube were averaged. A tube coordinate scale was established from 0 to 34 mm at the top and bottom of the tube, respectively. When filled to 1 ml, the meniscus is at 9.1 mm. The LDL profile was a plot of average NBD fluorescence intensity versus tube coordinate.

### SEC

LDL subfractions were applied to a fast-protein liquid chromatograph (Amersham Pharmacia, Inc.) equipped with two Superose HR6 columns in tandem and eluted with TBS buffer at a flow rate of 0.5 ml/min at room temperature. The elution profiles are presented as absorbance at 280 nm versus time.

### Apoptosis assays

Apoptosis assays were conducted as described previously (15, 25). Briefly, bovine aortic endothelial cells (BAECs) were maintained in DMEM supplemented with antibiotics, 1% L-glutamine, 1% penicillin-streptomycin, and 10% fetal bovine serum. For most of the experiments, cells (∼10⁶ at 8–10 passages) were seeded onto 96-well cell culture plates. Subconfluent cultures were switched to serum-free medium and incubated for 18 h with LDL subfractions, unfractionated NL-LDL, or PBS as a negative control. Cells were stained for 10 min with Hoechst 33342

(Molecular Probes, Eugene, OR) to assess nuclear morphology and with calcein acetoxymethyl ester and propidium iodide (Molecular Probes) to assess membrane integrity. Apoptosis was quantified according to the higher integrated pixel intensities produced by chromatin condensation. Nuclei were counted (500 cells/well) with MetaMorph (Universal Imaging Corp., West Chester, PA) in triplicate.

### Relative electrophoresis mobility by agarose gel

Twenty microliters of lipoprotein (5–10 μg of protein) was added to 5 μl of sample loading solution [40% sucrose and 0.05% bromophenol blue in tank/gel buffer (90 mM Tris, 80 mM borate, and 3 mM EDTA, pH 8.2)] (15). Twenty microliters was added to each well of a gel composed of 0.72% Gibco-BRL Life Tech Ultrapure Agarose in tank/gel buffer, followed by electrophoresis at 90 V at 4°C for 90 min. The gel was stained in Pierce GelCode blue reagent for 1 h and destained in deionized water.

### Statistical analyses

Results are expressed as means ± SD. Data were compared by unpaired two-tailed Student's *t*-tests for single comparisons or by one- or two-way ANOVA, with Student-Newman-Keuls or modified *t*-tests, respectively (SPSS 12.01), with differences deemed significant at $P < 0.05$.

### RESULTS

### LDL fractionation

In FH patients and NL subjects, respectively, the most electronegative LDL, L5, accounts for 2–5% and <0.6% of total plasma LDL. L1, the most abundant and electropositive subfraction, accounts for 70% and 90% (data not shown).

The density distribution of FH-LDL, FH-L1, and FH-L5 was analyzed by standard EDGUC using KBr (**Fig. 1**). The
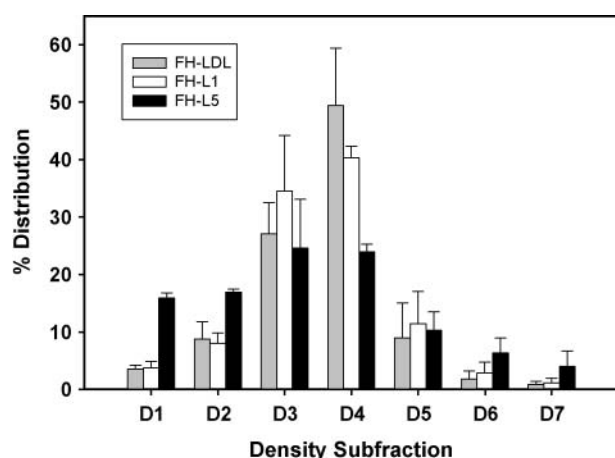
**Fig. 1.** Density distributions of LDL charged subfractions. Unfractioned familial hypercholesterolemic (FH)-LDL, FH-L1, and FH-L5 were obtained by separation into seven density subfractions in an equal volume (7 ml) by equilibrium density gradient ultracentrifugation with KBr solution at 40,000 rpm for 22 h. The distribution percentage was calculated based on optical density measurement. The density ranges for subfractions D1–D7 are as follows: D1, 1.035 ± 0.005 g/ml; D2, 1.038 ± 0.004 g/ml; D3, 1.042 ± 0.003 g/ml; D4, 1.046 ± 0.06 g/ml; D5, 1.050 ± 0.002 g/ml; D6, 1.055 ± 0.002 g/ml; D7, 1.064 ± 0.003 g/ml. Data represent means ± SD.

FH-LDL and FH-L1 distributed similarly, and the majority of LDL was contained in D3 (27.1 ± 5.4% for FH-LDL and 34.5 ± 9.7% for FH-L1) and D4 (50 ± 10% for FH-LDL and 40.3 ± 2.0% for FH-L1). Although the FH-L5 subfraction had qualitatively similar distributions, the relative amounts of both D3 and D4 were lower (24.6 ± 8.5% and 24.0 ± 1.3%, respectively), whereas the relative amounts at the extremes of the gradient, D1, D2, D6, and D7, were greater.

### Distribution of LDL subfractions by EDGUC in a NaBiEDTA gradient

Unfractionated LDL (three NL and three FH subjects) and five charged LDL subfractions (two NL and two FH subjects) were prestained with the fluorescent probe, NBD C$_6$-ceramide, and centrifuged in a self-generating NaBiEDTA gradient. The distribution of a typical unfractionated FH-LDL (**Fig. 2A**) appeared as an asymmetric bell-shaped curve with an apex at 1.030 g/ml. The breadth of the curve suggests considerable heterogeneity, and the small shoulder between 1.020 and 1.025 g/ml is indicative of a major underlying LDL subclass. The density distributions of five charged LDL subfractions of NL-LDL, L1–L5, were similar and also contained a prominent shoulder at the same position in the gradient.

In contrast, the density profiles of all charged LDL subfractions from FH patients (FH in Fig. 2B) were similar to each other but distinct from those of NL-LDL. FH-LDL distributed across a narrower range of densities than normal LDL. However, irrespective of whether the LDL was derived from FH or NL subjects, the LDL$^-$ was distributed across the entire range of densities. Thus, charge is not associated with a specific density range of LDL from NL or FH subjects; rather, charged LDL species are distributed across the entire range of densities for NL and FH-LDL.

### Size comparison by SEC

Differences in the sizes of various FH-LDL subfractions isolated according to density and charge were determined by SEC (Fig. 2C). According to the retention times, which increase with decreasing size, the most buoyant subfractions were associated with the largest LDL particles (FH-D1 elution volume, 21.61 ml), whereas the densest subfractions correspond to the smallest LDLs (FH-D7, 23.04 ml). The largest fraction of total LDL was found in D3 and D4, which eluted with retention times of 22.84 and 22.99 ml, respectively. In contrast, separation of FH-LDL by charge gave fractions with very similar elution volumes. FH-L1 and -L5, the most and least abundant of the variously charged FH-LDL subfractions, respectively, had elution volumes of 22.89 and 22.78 ml. All charged LDL subfractions were characterized by peak retention times between those of FH-D3 and -D4. Thus, the sizes of charged LDL subfractions are similar and independent of charge. Calculation of the size of LDL from its composition (17) and weighted partial specific volumes of each component (26) gives diameters ranging from 18.6 to 19.0 nm (**Table 1**).
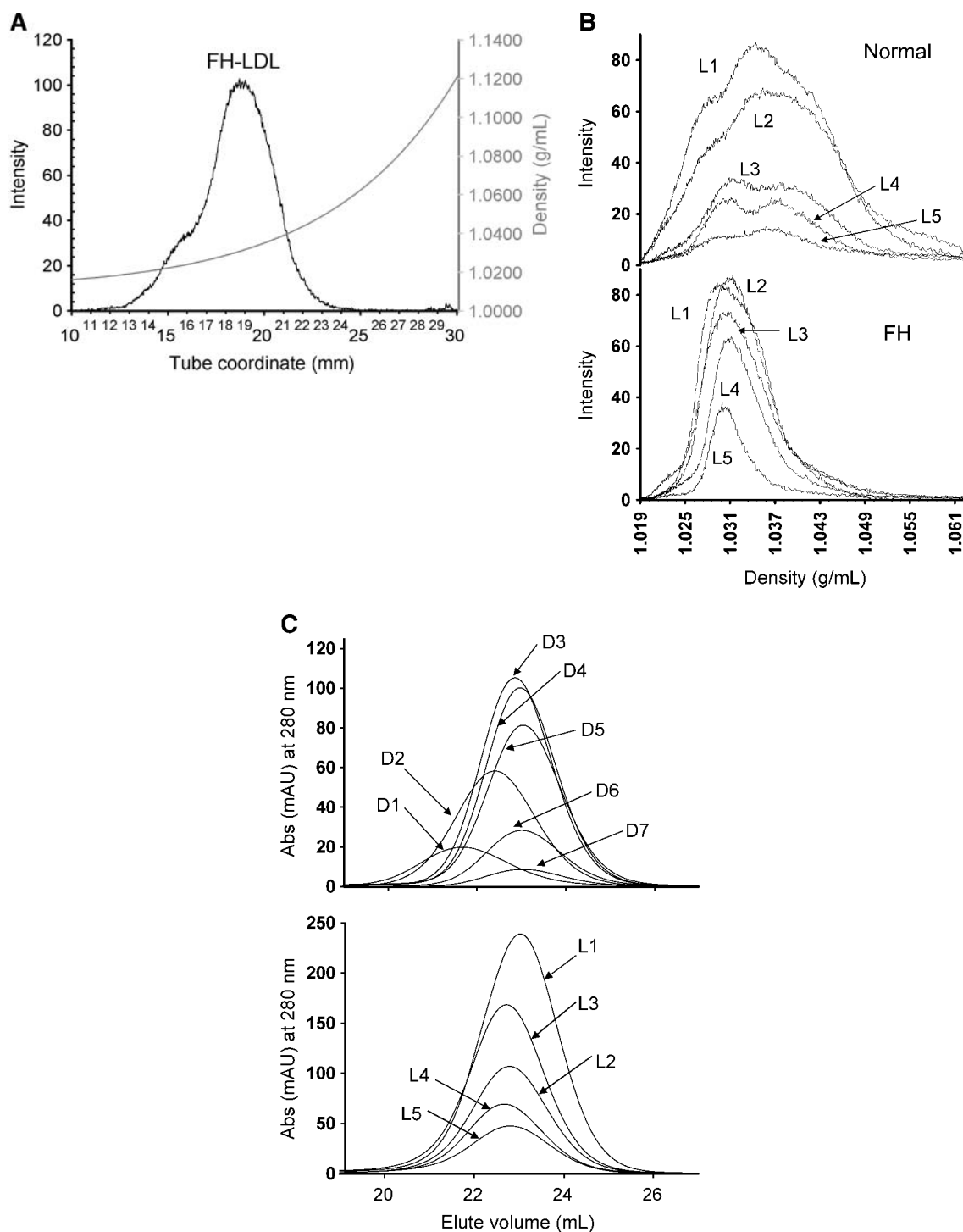
**Fig. 2.** Comparison of density distributions by equilibrium density gradient ultracentrifugation and of sizes by gel filtration chromatography for LDL charged subfractions. A: Representative density distribution of a FH-LDL sample was determined using rapid separation by sodium salt of a bismuth-EDTA complex gradient ultracentrifugation after incubation with 6-{[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]hexanoyl} (NBD $C_6$-ceramide). The calibration curve of density versus the tube coordinate was created by measuring the refractive index of 20 µl aliquots from discrete positions within the gradient. B: Density distributions of LDL charged LDL subfractions, L1–L5, for representative normolipidemic and FH subjects determined against the calibration curve. The LDL contents in each sample of 1 ml ranged from 20 to 80 µg based on protein contents. Fluorescence intensity was measured by digital imaging and analysis after 6 h of ultracentrifugation at 120,000 rpm. C: Size difference, determined by the elute volume, for FH density and charged subfractions compared using fast-protein liquid chromatography with Superose 6 columns. The curves for FH charged subfractions are shown at top and those for density subfractions are shown at bottom. Abs, absorbance; mAU, milliabsorbance units.

TABLE 1. Calculated sizes and experimental densities for charged
LDL subfractions from NL and FH subjects

| Subfractions | Size (Calculated) | Density (Calculated) | Density Apex |
|---|---|---|---|
| | $nm^3$ | $nm$ | $g/ml$ |
| NL | | | |
| L1 | 3,615.1 | 19.0 | 1.0303 |
| L2 | 3,495.3 | 18.8 | 1.0348 |
| L3 | 3,382.8 | 18.6 | 1.0377 |
| L4 | 3,586.8 | 19.0 | 1.0310 |
| L5 | 3,345.5 | 18.6 | 1.0312 |
| FH | | | |
| L1 | 3,570.5 | 19.0 | 1.0299 |
| L2 | 3,380.0 | 18.6 | 1.0299 |
| L3 | 3,424.7 | 18.7 | 1.0307 |
| L4 | 3,373.8 | 18.6 | 1.0305 |
| L5 | 3,567.9 | 19.0 | 1.0308 |

FH, familial hypercholesterolemic; NL, normolipidemic.

### Relationship between FH-LDL charge and apoptosis

BAECs were incubated with various concentrations of
FH-L5, and apoptosis was assessed according to the higher
integrated pixel intensities produced by chromatin con-
densation. These data showed that the proapoptotic effects
of FH-L5 were dose-dependent and linear up to at least
100 μg/ml (**Fig. 3A**, inset). BAECs were incubated with vari-
ous LDL subfractions, FH-L1 to -L5 (50 μg/ml protein),
and according to the same criteria, FH-L4 and -L5, respec-
tively, were highly proapoptotic, eliciting 10% ($P < 0.05$;
n = 3) and 40% ($P < 0.001$; n = 3) apoptosis, whereas in-
duction of apoptosis by FH-L1 to -L3 was <5% (Fig. 3A).
Comparison of the induction of apoptosis by the various
FH-LDL and -L5 components of each LDL density subfrac-
tion, D1–D7, showed that the most electronegative frac-
tion in each density class induced apoptosis, whereas the
FH-LDL fraction did not (Fig. 3B). The relative L5 content
of each density fraction, D1 through D7, as assessed from

the percentage absorbances appearing as L5 by ion-exchange
chromatography, were 11, 4.0, 4.0, 2.0, 2.5, 12, and 32%,
respectively. Induction of apoptosis by the same fractions,
D1 through D7, was linear with respect to their L5 content
($R^2 = 0.95$). The profound apoptotic effects associated with
higher L5 content suggested that electronegativity is more
closely linked to apoptosis than LDL size or density.

### Relative electrophoretic mobility on agarose gel

The electronegativity of FH-LDL, FH density subfrac-
tions (D1–D7), FH-L1 and -L5 were compared by agarose
gel electrophoresis (**Fig. 4**). Although density subclasses
were mixtures of charged subclasses, their relative elec-
trophoretic mobilities (REMs) appeared as a single band.
The REMs confirmed that FH-L5 and -L1 are the most and
least electronegative LDL species, respectively. Among FH-
LDL density subclasses, FH-D7 showed the greatest REM,
followed by FH-D6 and -D1, indicating that the extremes
of density are also electronegative.

### DISCUSSION

Although previous studies (21) revealed that the buoy-
ant and small, dense subfractions of FH-LDL were pref-
erentially enriched with LDL⁻, this has never been
systematically explored. According to gradient ultracen-
trifugation, SEC, and weighted partial specific volumes of
the components, L5, an LDL⁻, is not uniquely confined to
a narrow density or size range. In both FH and NL subjects,
all charged subfractions and native LDL share a similar
density pattern, in which their abundance correlates with
that of total LDL. Thus, the fractions with the highest LDL
content also have the highest L5 content, despite the frac-
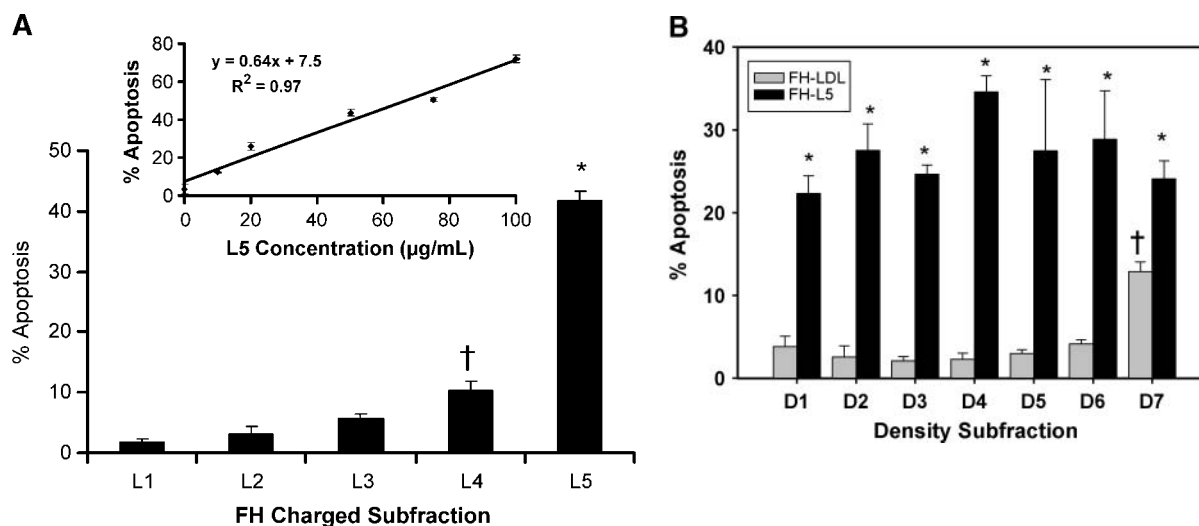tional contribution of L5 to the total being different from



**Fig. 3.** Apoptotic effects of FH-LDL charged subfractions. A: Apoptotic effects of FH-LDL charged subfractions (50 μg/ml) toward
endothelial cells. In the inset, a dose-dependent curve of the apoptotic effects (percentage) to the amount of FH-L5 up to 100 μg/ml gave a
calculated $R^2$ of 0.97 from the linear regression. Each of these panels and data points is representative of three experiments from one
individual subject. B: Comparison of apoptotic effects of density subfractions from unfractionated FH-LDL and FH-L5 at a 50 μg/ml base.
Data represent means ± SD. * $P < 0.001$, † $P < 0.01$ versus FH-LDL-D4, which showed the weakest activity among subfractions.
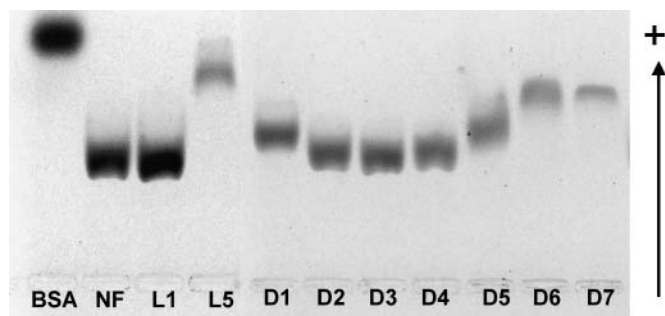
**Fig. 4.** Relative electrophoretic mobility (REM) on agarose gels. The REM comparison on a 0.72% agarose gel was for FH charged subfractions (L1 and L5) and FH-LDL density subfractions (D1–D7). Unfractioned FH-LDL (NF) and BSA were used as controls.

what is observed at the density extremes. L5 distributions in both FH and NL subjects were also broader than those of the more electropositive fractions. Because of its broader density distribution, L5 accounts for a greater percentage of total LDL at the buoyant and dense extremes of LDL density. A model (**Fig. 5**) shows density distribution constructed by Gaussian equations for native LDL, with which a curve for L5 accounting for 5% shares a similar density population pattern. A postulated greater standard deviation for L5 ($\sigma 2$) yields a broader Gaussian distribution than that for native LDL, with a standard deviation $\sigma 1$. The broader Gaussian distribution reflects the larger percentage of L5 at buoyant and dense subfractions of native LDL, whereas the percentage of L5 is lower in the most populated subfractions.

Although some have reported a correlation between sdLDL and CHD, this finding is confounded by coexisting hypertriglyceridemia and low HDL-cholesterol concentration (27, 28), both of which are CHD risk factors. There-
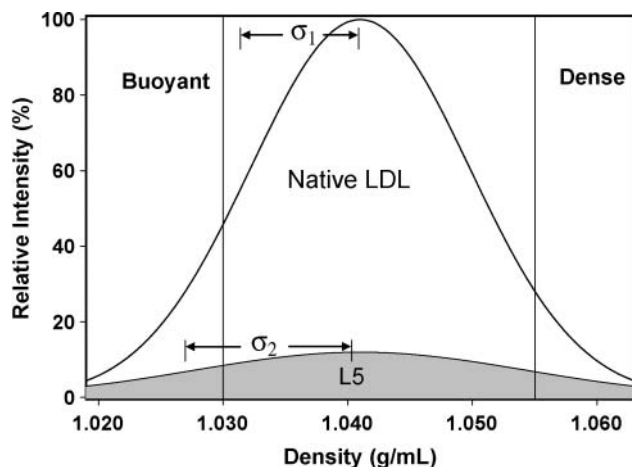


**Fig. 5.** Proposed density distribution model for the native LDL and L5 in plasma. Native LDL and L5 present with similar curve patterns constructed by a Gaussian equation, in which a greater standard deviation ($\sigma_2$) was given for L5 to form a broader distribution curve than that of native LDL, with a standard deviation $\sigma_1$. The broader distribution curve enhances the percentage of L5 in buoyant and dense subfractions of native LDL.

fore, LDL particle size is not an unambiguous CHD risk factor. In vitro studies have implicated modified LDL in the etiology of human atherosclerosis. Whereas in vitro studies of the biological activity of sdLDL are sparse, L5 induces apoptosis of BAECs (15). Several other model systems have been used to understand the mechanisms by which modified LDLs elicit their atherogenic effects, including metal ion oxidized LDL (29, 30) and minimally modified LDL (31). The physiological LDL subfractions that are mechanistically linked to atherosclerosis have been more difficult to identify with certainty, and much of the research focus has been on the effects of modified LDL on the biology of macrophages, a key cell type in several stages of atherogenesis. On the other hand, other initiating factors, including those that compromise the barrier properties of the arterial endothelium, also could be important. Thus, the proapoptotic properties of LDL$^-$, which is cytotoxic to endothelial cells (32), might be relevant to some stages of atherogenesis. Although statin therapy reduces total LDL and coronary events in a dose-dependent way (33), statins also reduce the plasma concentration of LDL$^-$ (34). Thus, LDL$^-$ could be one of several modified lipoprotein species that are atherogenic in vivo and that are amenable to statin therapy. Our data (Fig. 3B) revealed that apoptotic activity induced by all L5 density subfractions overwhelms that induced by native LDL density subfractions. Because sdLDL (D7) contains more L5 and its attendant biological activity, it could be concluded that classification of LDL according to charge is more closely linked to function than to size or density.

Our other key finding is that the magnitude of apoptosis induced by various LDL density subfractions is proportional to the amount of L5 in that fraction, irrespective of density. Moreover, this relationship held for density subfractions that had similar amounts of L5 but different amounts of total LDL. This is very important because it indicates that a small amount of L5 can elicit a biological effect within a background of normal LDL, and that normal LDL is not a competitive inhibitor of L5-induced apoptosis. This finding provides support for the hypothesis that L5 is proapoptotic under physiological conditions in which various amounts of normal LDL coexist and that dilution of L5 by other LDL fractions does not affect the magnitude of apoptosis. We conclude that L5 is a natural component of human plasma with the potential to initiate atherogenesis by compromising the integrity of the vascular endothelium.

The molecular basis of the association between LDL electronegativity and its cytotoxicity to endothelial cells, which may be independent of one another, is not known and may vary according to the identity of the underlying metabolic disorder. In addition, the biological effects of L5 may be attributable to a subpopulation with distinctive lipid or protein compositions and properties. Possible sources of these differences include apoprotein composition, triglyceride content, and protein conformation.

Lipoprotein charge is altered by lipid and protein composition and by the conformation of apoproteins on the lipoprotein surface. Conformational changes of apoB-100 could bury some charged amino acid residues, thereby

ablating their contributions to the total charge on the particle. Differences in the charge on HDL with preβ and preα mobility have been attributed to differences in protein conformation and phosphatidylinositol content (35). Presumably, differences in the exposure of charged amino acids could alter their charge and that of the total particle. In type 1 diabetic patients, electronegative and electropositive LDLs are distinguished by higher contents in the former of triglyceride, nonesterified fatty acids, apoE, apoC-III, and platelet-activating factor acetylhydrolase, differences that are independent of oxidation and glycosylation (36). The composition of LDL⁻ from FH patients is similarly altered (17). The calculated isoelectric points of 5.65 and 5.23 for apoE and apoC-III, respectively, could contribute to electronegativity, because the calculated isoelectric point for apoB-100 is 6.61.

Although some data show that the properties of the surface lipids of LDL⁻ are different from those of normal LDL (17, 37), these differences are likely to be transmitted from the core, which is more triglyceride-rich in LDL⁻. Of the compositional differences, the TG content might be expected to elicit the greatest differences both functionally and structurally. TG-rich LDL from hypertriglyceridemic patients or prepared from normal LDL in vitro is electronegative and recognized differently by the LDL receptor, effects that may be attributable to the observed alterations of apoB-100 conformation (38, 39) and the profound differences in the structure and properties of the neutral lipid core (26).

The different results by ion-exchange chromatography and agarose gel electrophoresis can be explained by the exchangeable character of the charged species in LDL and by differences in the way the two techniques separate molecules and/or particles. In agarose gel electrophoresis, the particles pass through the gel without binding, so that exchange occurs readily. In contrast, when whole LDL is loaded onto an ion-exchange column in starting buffer, each particle binds to a specific site on the stationary phase, so there is minimal interparticle interaction. As the ionic strength is increased, those particles with the least electronegativity are released first and exit the column, and the most electronegative fractions are released last and pass through the mobile phase as the sole species, with no other species with which to interact or transfer charge components. According to an exchange model, whole LDL, which is composed of L1 to L5, would be expected to migrate as some average of the migration behavior of its components.

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