Density distribution of electronegative LDL in normolipemic and hyperlipemic subjects

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Abstract The density distribution of electronegative LDL [LDL(−)], a cytotoxic and inflammatory fraction of LDL present in plasma, was studied in 10 normolipemic (NL), 6 FH, and 11 hypertriglyceridemic (HTG) subjects. Six LDL subclasses of increased density (LDL1 to LDL6) were isolated by density-gradient ultracentrifugation (DGU). NL and FH subjects showed prevalence of light LDL, whereas HTG subjects showed prevalence of dense LDL. LDL(−) proportion was determined from total LDL or LDL-density subclasses by anion-exchange chromatography. LDL from FH patients had increased LDL(−) (35.1 ± 9.9%) compared with LDL from NL and HTG subjects (9.4 ± 2.3% and 12.3 ± 4.3%, respectively). Most LDL(−) was contained in dense subclasses in NL (LDL4–6, 67.7 ± 3.1%) whereas most of LDL(−) from FH patients were contained in light LDL subclasses (LDL1–3) (86.2 ± 1.6%). In these subjects, simvastatin therapy decreased LDL(−) to 28.2 ± 6.7% and 21.2 ± 5.6% at 3 and 6 months of treatment, respectively, due mainly to decreases in light LDL subclasses. In HTG subjects, half LDL(−) was contained in dense LDL subclasses (LDL4–6, 46.1 ± 2.0%). Non-denaturing acrylamide gradient gel electrophoresis concurred with DGU data, as LDL(−) from NL showed a single band of lower size than non-electronegative LDL [LDL(+)], whereas LDL(−) from FH and HTG presented bands of greater size than its respective LDL(+). These results reveal the existence of light and dense LDL(−), indicate that hyperlipemia could promote the formation of light LDL(−) and suggest that LDL(−) could have different origins.—Sánchez-Quesada, J. L., S. Benítez, C. Otal, M. Franco, F. Blanco-Vaca, and J. Ordóñez-Llanos. **Density distribution of electronegative LDL in normolipemic and hyperlipemic subjects. *J. Lipid Res.* 2002.** 43: **699–705.**

Supplementary key words modified LDL • LDL density subclasses • hyperlipemia

Growing evidence suggests that qualitative modifications in LDL are related to atherosclerosis development (1). Several modifications increasing the negative charge of LDL are able to induce cholesteryl ester accumulation in macrophages and subsequent foam cell formation (2). Oxidative modification appears to play a key role in the atherogenicity of LDL, although other modifications occurring in vivo, such as glycation or desialylation, have also been related to atherogenesis (3, 4). Oxidized LDL (oxLDL) presents several atherogenic properties, including cytotoxicity for endothelial cells, induction of the expression of cytokines, vascular adhesion molecules, tissue factor and plasminogen-activator inhibitor, and inhibition of nitric oxide synthesis (1, 2, 5). It is generally thought that oxLDL is produced when LDL enters the endothelium and is trapped in the arterial wall intima (1). However, by anion exchange chromatography several groups isolated a subfraction of LDL with increased negative charge in plasma (6–14) that has been described as cytotoxic (9, 13) and proinflammatory in cultured endothelial cells (14). Some authors suggested that this electronegative LDL [LDL(−)] could represent the in vivo counterpart of in vitro mildly oxidized LDL, as they found increased lipid hydroperoxides, conjugated dienes, and oxidized cholesterol, and decreased α-tocopherol and polyunsaturated fatty acids (6–11). However, the oxidative origin of LDL(−) has been discussed by other authors as they found no evidence of oxidative modification and attributed the increased negative charge to a higher content of sialic acid, apolipoprotein E (apoE), apoC-III, or free fatty acids (12–16). The distribution of LDL(−) throughout the LDL density subclasses is also a matter of controversy, as a preferential distribution of LDL(−) in the most dense LDL subfractions (8) or a bimodal distribution with both light and dense subfractions have been described (12). It is well established that the abnormal abundance of small, dense LDL particles, commonly known as phenotype B of LDL density subclasses, is related to increased cardiovascular risk (17). Thus, the possible association be-

Abbreviations: DGU, density gradient ultracentrifugation; FPLC, fast protein liquid chromatography; GGE, gradient gel electrophoresis; HTG, hypertrigliceridemic; LDL(−), electronegative LDL; NL, normolipemic.

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tween increased electronegativity and increased density could contribute to increased atherogenicity of these particles. The aim of the current work was to evaluate the proportion of LDL(−) and its density distribution in subjects showing LDL phenotype A (predominance of large, light LDL particles), including healthy normolipemic and familial hypercholesterolemic subjects, and phenotype B hypertriglyceridemic subjects.

MATERIALS AND METHODS

Subjects

Ten normolipemic (NL), six FH, and nine moderate hypertriglyceridemic (HTG) subjects were analyzed. FH patients were diagnosed on the basis of MedPed criteria (18) (family history, xanthomata, total cholesterol >9 mmol/l, LDL cholesterol (LDL-C) >7 mmol/l). HTG patients presented moderately increased triglyceride concentrations (from 1.94 to 4.00 mmol/l) based on reference values derived from a Spanish population (19). A major inclusion criterion was the predominance of light or dense LDL subclasses determined by gradient density ultracentrifugation (DGU), as described in Materials and Methods. Thus, NL and FH subjects showed predominance of large, light LDL subclasses (phenotype A), whereas HTG presented predominance of small, dense LDL subclasses (phenotype B). Hyperlipemic patients were free of lipid-lowering drugs for at least 1 month for statins and 2 months for fibrates prior to the study. FH subjects were also analyzed after 3 and 6 months of therapy with 40 mg/day of simvastatin. All subjects gave written informed consent according to the ethics guidelines of our hospital.

Lipid analysis

Plasma samples were obtained from venous blood collected in EDTA-containing Vacutainer tubes. Total cholesterol was determined by an enzymatic method (CHOD-PAP, Roche, Basel, Switzerland). Cholesterol fractions were quantified by combined ultracentrifugation-precipitation method utilizing Cl_2Mg-phosphotungstate as the precipitating reagent, as recommended by the Lipid Research Clinics Program (20). Plasma triglyceride was measured by an enzymatic method (GPO-PAP, Roche). Plasma Lp[a] levels were measured by ELISA (Apo-Tek Lp[a], Sigma Diagnostics, Saint Louis, USA).

Total LDL isolation

Plasma was isolated by centrifugation at 1,500 *g* for 15 min at 4°C from venous blood drawn in EDTA-containing Vacutainer tubes. Fresh total LDL (density 1019–1063 g/l) was isolated by sequential flotation ultracentrifugation (21). Oxidative modifications during lipoprotein isolation were minimized using degassed KBr solutions containing 1 mM EDTA and ultracentrifugation at 4°C.

LDL density subclasses

LDL density subclasses were isolated from plasma-EDTA by DGU, as described (22). Briefly, 6 LDL subclasses, namely LDL1 (mean density 1,022 g/l), LDL2 (1,027 g/l), LDL3 (1,032 g/l), LDL4 (1,039 g/l), LDL5 (1,047 g/l), and LDL6 (1,056 g/l) were isolated in 0.8 ml aliquots. The [(LDL1+LDL2+LDL3)/(LDL4+LDL5+LDL6)] ratio was expressed as the percentage of each LDL subclass cholesterol with respect to total LDL-C. Ratios higher than 1.8 were considered phenotype A and lower than 1.1 phenotype B (23). The relative content in total and free cholesterol (Roche), triglyceride (Roche), phospholipid (Wako, Neuss, Germany), and protein (Bio-Rad, Munchen, Germany) was evaluated in each LDL subfraction and results were expressed as the percentage of total mass.

Quantification of LDL(−)

LDL(−) was isolated by anion exchange chromatography (AEC) (column Mono Q 5/5, Pharmacia, Uppsala, Sweden) in a Fast Protein Liquid Chromatography (FPLC) system (Pharmacia) (22, 24) from total LDL (1019–1063 g/l) or from LDL subfractions obtained after DGU. Prior to AEC, all samples were dialyzed against buffer A (10 mM Tris, 1 mM EDTA, pH 7.4) by gel filtration chromatography (Sephadex G-25M, Pharmacia). Two LDL forms, named LDL(+) (elution at 0.2 M NaCl) and LDL(−) (elution at 0.3 M NaCl) were identified at 280 nm and their relative proportion quantified by peak integration. In some experiments, LDL(+) and LDL(−) were collected in 1 ml fractions, concentrated by ultracentrifugation, and analyzed by acrylamide gradient gel electrophoresis, as described later.

Non-denaturing acrylamide gradient gel electrophoresis

LDL particle size was determined by GGE (2–16%) according to Nichols et al. (25) with modifications. Two solutions at 2% and 16% were prepared using a stock solution of acrylamide and bisacrylamide (30% total, 5% cross linker), and mixed using two P-1 peristaltic pumps (Pharmacia). LDL(+) and LDL(−) fractions (10 μl at 0.5–1 g/l) from each group of patients were pre-incubated for 15 min with 10 μl of Sudan black (0.1% w/v in ethyleneglycol), and 5 μl of saccharose (50% w/v). Ten microliters of this mixture were electrophoresed at 4°C for 30 min at 20 V, 30 min at 70 V, and 16 h at 100 V. Bands were scanned by densitometry at 595 nm and LDL size was determined using a plasma pool containing four LDL bands of known size (22.9 ± 0.5, 24.5 ± 0.2, 26.2 ± 0.2, and 28.4 ± 0.4 nm) as a standard. Diameter of standard LDL bands was previously assessed by electron microscopy.

Statistical analysis

Inter-group differences were evaluated with the Mann-Whitney U test. Differences between LDL subfractions of each group were tested with Wilcoxon's *t*-test. Association between variables was tested by the Spearman ordinal correlation. $P < 0.05$ was considered statistically significant. Results are expressed as mean ± SD.

RESULTS

Gender, age, and lipid profile of all studied subjects are shown in **Table 1**. Lipoprotein profile of hyperlipemic patients was characteristic of FH and moderate hypertriglyceridemia. After simvastatin therapy, total, LDL-C, and VLDL-C diminished significantly in FH subjects ($P < 0.05$) without changes in triglyceride and HDL-C HTG subjects showed an LDL density subclass ratio of 1.04 ± 0.42. NL subjects and FH patients presented prevalence of large, light LDL subclasses (NL ratio: 2.52 ± 0.49; FH ratio: 3.62 ± 0.42). Ratios of HTG and FH subjects were significantly lower and higher, respectively, than that of NL subjects ($P < 0.05$). Plasma Lp[a] was lower than 100 mg/l in all subjects included in the study.

Total LDL composition is shown in **Table 2**. Protein content in LDL from HTG patients was higher than in LDL from NL and FH, reflecting the lower LDL particle size in these subjects. In addition, triglyceride was higher and phospholipid lower in HTG subjects compared with

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TABLE 1. Anthropometric characteristics and lipoprotein profile of NL, FH, and HTG subjects

	NL	FH (0 months)	FH (6 months)	HTG
Males/Females	5/5	3/3		5/6
Age (years)	36.5 ± 5.1	47.0 ± 8.3		45.1 ± 9.2
BMI (kg/m^2)	22.2 ± 1.7	24.6 ± 2.5		24.5 ± 1.8
Total cholesterol (mmol/l)	4.94 ± 0.72	11.02 ± 1.82[a]	7.25 ± 0.97[a,b]	5.73 ± 0.97[b]
Triglycerides (mmol/l)	0.69 ± 0.19	1.38 ± 0.66[a]	1.14 ± 0.98	2.15 ± 0.83[a,b,c]
VLDL-C (mmol/l)	0.30 ± 0.12	0.55 ± 0.47	0.41 ± 0.19[b]	0.78 ± 0.38[a,b,c]
LDL-C (mmol/l)	3.03 ± 0.42	9.24 ± 1.65[a]	5.77 ± 1.01[a,b]	3.83 ± 0.89[a,b,c]
HDL-C(mmol/l)	1.69 ± 0.47	1.24 ± 0.32[a]	1.37 ± 0.43	1.12 ± 0.18[a]

Concentrations are expressed as mean ± SD.
[a] Statistically significant differences versus NL subjects.
[b] Statistically significant differences versus FH subjects before simvastatin therapy.
[c] Statistically significant differences versus FH subjects after simvastatin therapy.

NL and FH subjects. On the other hand, LDL from FH patients was enriched in esterified cholesterol compared with NL and HTG subjects. Simvastatin therapy did not change LDL composition in the FH group (data not shown).

The composition of LDL subclasses in the three studied groups shared several common features (**Fig. 1**). As expected, the proportion of protein increased and cholesterol decreased with density. Free cholesterol content was lower in dense LDL subclasses. Triglyceride was lower in those of intermediate density (LDL3–4). LDL subclasses of HTG patients had lower phospholipid (LDL1 to 6) and higher triglyceride content (in subclasses LDL1–2 and LDL4–6) than those of FH and NL subjects. In addition, LDL1–3 of HTG contained less, and LDL5–6 more cholesterol than the same subclasses of FH and NL subjects. On the other hand, all LDL subclasses of FH patients had higher cholesterol and lower triglyceride content than NL subjects. All these differences were statistically significant ($P < 0.05$). Simvastatin therapy did not modify LDL subclass composition (data not shown). LDL density subclasses were tested for Lp[a], as this lipoprotein is known to be electronegative; however, Lp[a] was undetectable in all subfractions.

The proportion of LDL(−) obtained in NL subjects (9.4 ± 2.3%, range 5.7–12.2%) concurs with results previously published by our group (14, 22, 26). Although HTG patients presented higher LDL(−) proportion than NL subjects (12.3 ± 4.3%), the wide range observed (5.5–18.1%) prevented statistical differences compared with NL subjects. On the other hand, FH patients showed a very high proportion of LDL(−) (35.1 ± 9.9%, range 26.0–49.2%), higher than that observed in NL and HTG subjects ($P < 0.05$). LDL(−) proportion was significantly reduced to 28.2 ± 6.7 at 3 months and to 21.2 ± 5.6% at 6 months ($P < 0.05$) of simvastatin therapy in FH patients. When LDL(−) proportion of all subjects was considered, statistically-significant correlations with plasma cholesterol and LDL-C ($r = 0.715$ and $r = 0.778$, respectively, $P < 0.05$) were observed; however, these associations disappeared when each group was individually analyzed.

TABLE 2. Composition of total LDL from NL, FH, and HTG subjects

	NL	FH	HTG
Total cholesterol	44.28 ± 0.60	46.18 ± 1.80[a]	44.25 ± 3.04[b]
Free cholesterol	12.03 ± 0.10	12.36 ± 1.10	11.05 ± 2.54
Phospholipid	32.65 ± 0.63	31.86 ± 0.85	28.88 ± 0.93[a,b]
Triglyceride	6.55 ± 1.27	5.85 ± 1.42	8.58 ± 2.64[a,b]
Protein	16.38 ± 0.10	16.07 ± 1.63	20.05 ± 1.22[a,b]

Results are expressed as % of total LDL mass (mean ± SD).
[a] Statistically significant differences versus NL subjects.
[b] Statistically significant differences versus FH subjects.

The proportion of LDL(−) in each LDL density subclass is shown in **Fig. 2A**. In NL subjects, LDL(−) was relatively abundant in the most dense LDL subclasses (18.2 ± 5.5%, 45.6 ± 12.2%, and 80.4 ± 6.9% in LDL4 to 6, respectively), but accounted for less than 8% in large, light subclasses (LDL1–3). HTG patients showed a different pattern from NL subjects, as although LDL(−) was abundant in LDL5 and LDL6 subclasses (21.2 ± 8.3% and 45.9 ± 8.1%), its relative concentration was also high in LDL1 and LDL2 subclasses (32.8 ± 15.7% and 18.0 ± 6.4%). Finally, FH individuals differed from NL and HTG subjects, as the proportion of LDL(−) was relatively abundant in all density subclasses (higher than 20%), principally in the two lightest (53.8 ± 10.7%, 34.0 ± 5.9% in LDL1 and LDL2, respectively). Figure 2B shows the effect of simvastatin at 3 and 6 months of treatment in FH patients on the proportion of LDL(−) contained in each LDL density subclass. The effect of simvastatin was more intense in the light LDL subclasses (a decrease of approximately 50% in LDL1–3) than in the dense subclasses (decrements ranged from 30% in LDL4 to only 5% in LDL6).

The amount of total LDL and LDL(−) contained in dense LDL subclasses (LDL4–6) is shown in **Table 3**. In NL subjects, 67.7 ± 3.1% of total LDL(−) was contained in dense subclasses. However, the distribution pattern of LDL(−) in FH patients resembled the distribution of total LDL, being only 13.8 ± 3.1% of LDL(−) contained in dense LDL subclasses (LDL4–6). In HTG patients, 48.1 ± 2.0% of LDL(−) was contained in LDL4–6. These data showed that most LDL(−) are small, dense particles in the NL group and most LDL(−) are large, light particles in FH patients, whereas HTG subjects present both dense and light LDL(−) particles.

Results obtained with GGE concur with data obtained from DGU. **Figure 3** shows a representative electrophore-

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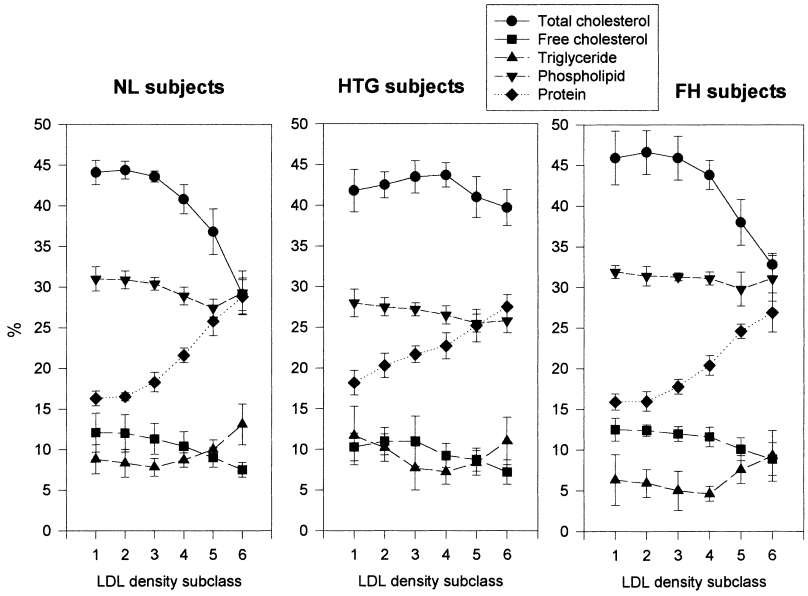

Fig. 1. Lipid and protein composition of the six LDL density subclasses in normolipemic (NL), hypertriglyceridemic (HTG), and FH subjects.

sis of LDL(+) and LDL(−) isolated from NL, FH, and HTG subjects. LDL(−) from NL subjects showed a single band (25.0 ± 0.2 nm measured in three independent experiments) of smaller size than the LDL(+) subfraction (26.0 ± 0.1 nm). In contrast, LDL(−) from FH patients showed the presence of a large-sized bands (higher than 28.4 nm) and the most intense band had a similar size to LDL(+) (26.2 ± 0.3 nm). Concerning HTG patients, LDL(+) was smaller (24.5 ± 0.3 nm) than LDL(+) from NL and FH subjects, and LDL(−) presented three bands, one with the same size as its LDL(+) (24.4 ± 0.3 and two bands with larger size (26.0 ± 0.2 and 28.0 ± 0.4 nm), suggesting the presence of both small and large LDL particles.

DISCUSSION

The role of oxLDL in atherogenesis has been proven by many in vitro and in vivo evidences (1–5). Accordingly, the existence in plasma of a subfraction of oxidized, even mildly oxidized LDL, could constitute a marker of the atherogenic process. However, the abundance of antioxidant molecules present in plasma led most investigators to argue against the presence of such a hypothetical lipoprotein in the blood stream. Thus, major attention has been focused on the study of the oxidation of LDL trapped in the arterial wall. The identification by Avogaro of a plasma LDL(−) subfraction raised the possibility that this lipoprotein could represent the in vivo counterpart of in vitro

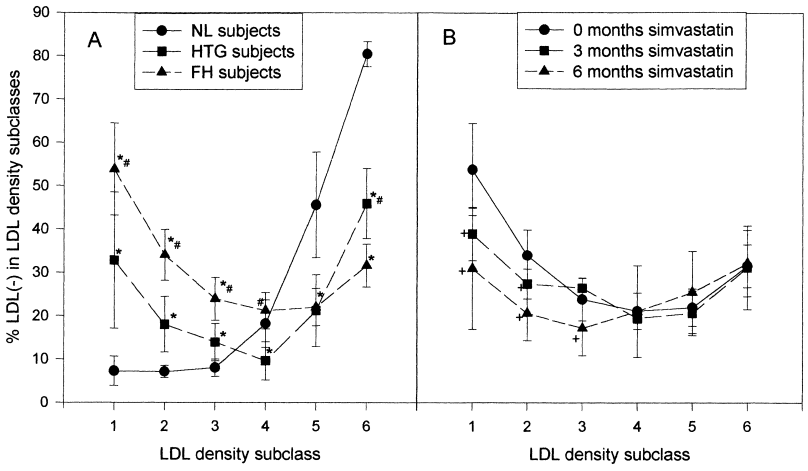

Fig. 2. A: Percentage of LDL(−) in each of the six LDL density subclasses (LDL1–6) obtained by gradient density ultracentrifugation in NL, HTG, and FH subjects. B: Effect of simvastatin therapy on the percentage of LDL(−) in FH subjects. $^{*}P < 0.05$ versus NL subjects; $^{\#}P < 0.05$ versus HTG subjects; $^{+}P < 0.05$ versus 0 months of simvastatin therapy.

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TABLE 3. Percentage of total LDL and LDL(−) contained in the dense LDL subfractions (LDL4–6)

	NL	FH	HTG
Total LDL	20.99 ± 2.41	13.88 ± 1.17[a]	56.50 ± 1.39[a,b]
LDL(−)	67.72 ± 3.09[c]	13.78 ± 1.57[a]	46.14 ± 2.03[a,b,c]

[a] Statistically significant differences versus NL subjects.
[b] Statistically significant differences versus FH subjects.
[c] Statistically significant differences versus total LDL.

mildly oxidized LDL (6). Since then, several authors have isolated from plasma an electronegatively-charged LDL subfraction, which has been denominated LDL− (6–11, 27), LDL(−) (12−15, 22, 26, 28), minor LDL (16,29), or modified LDL (30). However, discrepancies have arisen in the amount, ranging from 1% to 10% of total LDL, and also in the origin and characteristics of LDL(−) in NL subjects. The characteristics described in different studies by Avogaro and co-workers suggested an oxidative origin for LDL(−), as a higher content in lipoperoxides and cholesterol oxides and lower content of vitamin E and polyunsaturated fatty acids were reported (6–11). Recently, it has been described that LDL(−) presents epitopes recognized by antibodies developed to MDA-modified and 4-hydroxynonenal-modified LDL (29, 30). Nevertheless, other authors found no evidence of oxidative modification in LDL(−) (12–16, 24), and the increase in its negative charge was attributed to a higher content of apoE and C-III, sialic acid, and non-esterified fatty acids (12–15). Despite the discrepancies reported in the physicochemical characteristics of LDL(−), the authors concur on the atherogenicity of these particles, as cytotoxic (9, 13) and inflammatory (14) effects have been described in cultured endothelial cells. It is noteworthy that even when no evidence of oxidative modification was found in LDL(−), it was cytotoxic (13) and induced the production of interleukin-8 and monocyte chemotactic protein-1 in endothelial cells (14). Discrepancies among authors could be due to differences in methodological conditions, but a possible heterogeneity in the characteristics of LDL(−), specifically in its size and density, as well as in the subjects from which is isolated, may also explain some of these discrepancies. Evidence obtained in the current work supports this assumption.

LDL is a heterogeneous group of particles that differ in size, density, electric charge, and composition. According to size and density, two main phenotypes of plasma LDL have been identified. The abnormal abundance of small, dense LDL particles, known as phenotype B, confers an increased cardiovascular risk three times higher than that observed in subjects with phenotype A (predominance of large, light LDL particles) (17). Small, dense LDL are more prone to oxidation and present lower affinity for LDL receptor than large, light LDL (31–33). These characteristics have also been attributed to LDL(−), which has been described to be, at least partially, contained in dense LDL subclasses (8). This reasoning suggests that subjects with phenotype B should present a high proportion of LDL(−), whereas phenotype A subjects should have low amounts of these particles. However, FH subjects, known to present phenotype A, showed a proportion of LDL(−) 4-fold higher than the normal population, which decreased after simvastatin therapy (28). Morever, insulin-dependent diabetic subjects with phenotype A showed a high LDL(−) proportion that decreased after insulin therapy (22). In both studies, total LDL oxidizability and LDL subclass phenotype did not differ from those of control group and were not affected by simvastatin or insulin treatment. These observations suggest that in some groups of subjects with a high proportion of LDL(−), these particles could also be contained in large, light LDL subclasses.

The proportion of LDL(−) in NL (14, 22, 26, 28) or FH subjects (28) concurs with previous data obtained by our group. HTG subjects with phenotype B presented a wide range of LDL(−) proportion, perhaps reflecting differences in the cause of their hypertriglyceridemia. The distribution of LDL(−) in NL subjects indicates that this lipoprotein is distributed preferentially in the most dense LDL subclasses, with more than 67% of LDL(−) being contained in dense LDL (LDL4–6). This observation is in accordance with results reported by other authors (8, 31) and links, in these particles, two LDL characteristics that are potentially atherogenic, of high density, and increased electronegativity.

In contrast, more than 85% of LDL(−) in FH subjects was contained in the lightest LDL subclasses. The LDL(−) lowering action of simvastatin was exerted mainly on light, rather than dense, LDL subclasses. The finding that simvastatin therapy, which increases LDL receptor expression, dramatically decreased the proportion of LDL (−) in light, but not in dense, subclasses suggests that the impairment in the VLDL-IDL-LDL catabolic cascade due to the lack of functional LDL receptors could play a major role in the production of light LDL(−), and in the different distribution of these particles compared with the NL population. These results would explain previous results in which a great decrease in LDL(−) after simvasta-

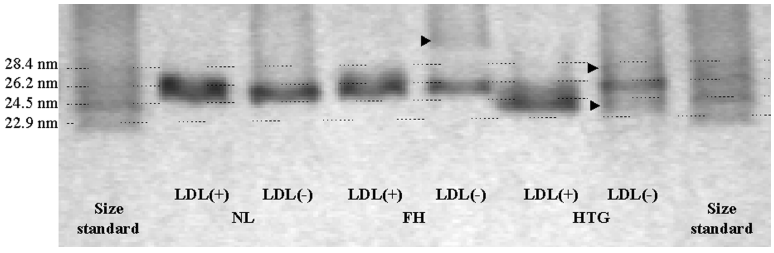

Fig. 3. Polyacrylamide gradient gel electrophoresis of LDL(+) and LDL(−) fractions isolated from total LDL by anion exchange chromatography from NL, HTG, and FH subjects. Lane 1: LDL size standard; lane 2: LDL (+) from NL; lane 3: LDL(−) from NL; lane 4: LDL(+) from FH; lane 5: LDL(−) from FH; lane 6: LDL(+) from HTG; lane 7: LDL(−) from HTG; lane 8: LDL size standard. Arrows indicate large-sized band (higher than 28.4 nm) in LDL(−) from FH, and 28.0 nm and 24.4 nm bands in LDL(−) from HTG subjects.

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tin therapy was not accompanied by changes in LDL susceptibility to oxidation (28), since light LDL(−) could not be more susceptible to oxidation, in contrast to that described for dense LDL(−) in NL subjects (8). Differences between NL and FH subjects suggest that physicochemical characteristics of LDL(−) and, consequently, the origin of electronegative charge could be different in both groups. It is worth noting that NL and FH subjects present LDL density subclass phenotype A. This indicates that, beyond the LDL subclass pattern, the presence of light or dense LDL(−) particles depends on the presence of a pathological dysfunction.

Moderate HTG patients showing phenotype B presented an LDL(−) distribution with both light and dense LDL(−) particles. Thus, one half of LDL(−) in the HTG group is contained in the light LDL subclasses (LDL1–3) and one half in the dense subclasses (LDL4–6). Whether LDL(−) from HTG subjects resembles the characteristics of LDL(−) from FH and LDL(−) from NL subjects, respectively, is unlikely, as most LDL(−) from HTG subjects was contained in middle-density subclasses (LDL3–4).

The density distribution of LDL(−) observed using DGU was confirmed by GGE. Thus, LDL(−) from NL subjects showed a single band that was smaller than its respective LDL(+). LDL(−) from FH subjects presented two bands, one with the same size as LDL(+) and other of larger size; this second band could be related to the high proportion of LDL(−) observed in LDL1–2 subclasses. Concerning HTG subjects, LDL(−) showed three bands including both large, medium, and small particles; this heterogeneity could explain the presence of considerable amounts of LDL(−) in all density subclasses in HTG patients.

The composition of total LDL was characteristic of each group of subjects. LDL from FH subjects was relatively enriched in cholesterol, whereas LDL from HTG was enriched in protein and triglyceride and depleted in phospholipid compared with NL subjects. With respect to the composition of each LDL density subclass, some characteristics were common to the three studied groups. Thus, protein increased with density whereas total and free cholesterol decreased. Lighter (LDL1–2) and denser (LDL5–6) subclasses contained a higher percentage of triglyceride than LDL3–4. These data concur with previously published data (34) and with the observation that LDL(−) was relatively abundant in denser or lighter LDL density subclasses, as LDL(−) was reported to have increased triglyceride content (6–11, 14).

Our finding that LDL(−) is more abundant in light and dense LDL subclasses concurs with data of La Belle et al. (34) and Lund-Katz et al. (35) who demonstrated by electrophoresis that light and dense LDL subclasses have more surface charge and electrophoretic motility than those of intermediate density.

We conclude that LDL(−) shows different density distribution in NL, FH, or HTG subjects. Most LDL(−) in NL is contained in the dense LDL subfractions, whereas most LDL(−) in FH is contained in the light LDL subfractions. In contrast, HTG subjects show LDL(−) in dense, intermediate, and light particles. These results suggest that LDL(−) is a heterogeneous group of particles that share as a common feature an increased negative charge, but have different physicochemical characteristics and, probably, different origins. However, studies on endothelial function were developed with LDL(−) isolated from NL subjects (9, 13, 14), and it is not known whether large, light LDL(−) isolated from dyslipemic subjects would exert the same effects on endothelial cells. In this respect, it would be interesting to evaluate separately the chemical characteristics and the effect on cells of dense and light LDL(−) isolated from different groups of dyslipemic subjects. Further studies are required to evaluate the atherogenic characteristics of LDL(−) isolated from dyslipemic subjects and to characterize in detail dense and light LDL(−).

This work was supported by grants from Comisión Interministerial de Ciencia y Tecnologia of the Ministerio de Investigación y Ciencia (SAF98/0125 to J.O-L.), Laboratorios Fournier (to J.L.S-Q.) and Merck, Sharp & Dohme (to J.O-L., M.F., and J.L.S-Q.). S.B. is a recipient of a Comissió Interdepartamental de Recerca i Innovarió Technologia predoctoral fellowship from Generalitat de Catalunya. The authors are grateful to Amalia Payés and Laia Bayén for technical assistance and to Christine O'Hara for editorial assistance.

Manuscript received 25 June 2001 and in revised form 1 November 2001.

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