Abstract
The triglyceride content of the plasma very-low-density lipoprotein (VLDL) fraction is the most important factor affecting the size of low-density lipoprotein (LDL) in humans. Because cholesteryl ester transfer protein (CETP) can influence the size distribution of LDL particles in human plasma, the implication of lipid transfers in the formation of small-sized LDL patterns, which have been associated with elevated plasma triglyceride levels, was investigated. The size distribution of LDL particles in 15 plasma samples was determined by electrophoresis of the plasma LDL fraction on 20 to 160 g/L polyacrylamide gradient gels. The apparent diameter of the major LDL subfraction was shown to correlate negatively with triglyceride concentrations \((r = -0.706, P < 0.005)\) and positively with both high-density lipoprotein cholesterol levels \((r = 0.637, P < 0.02)\) and the high-density lipoprotein/VLDL + LDL cholesterol ratio \((r = 0.768, P < 0.001)\). In addition, LDL size correlated negatively with both the ability of plasma LDL to donate cholesteryl esters \((r = -0.79, P < 0.001)\) and its ability to acquire triglycerides \((r = -0.72, P < 0.005)\). Whereas these observations indicated that CETP-mediated alterations of the triglyceride/cholesteryl ester ratio of the LDL core would contribute to changes in LDL diameter, they suggested that the formation of small-sized gradient gel LDL patterns would require another biochemical event, such as lipolysis, in addition to neutral lipid transfers. To test this hypothesis, total plasma samples with or without added VLDL (added triglyceride concentration, 2.0 g/L) were preincubated for 24 hours at 37°C. Preincubation mainly induced the replacement of cholesteryl esters by triglycerides in the LDL core, and changes in LDL composition were greater when total plasma was supplemented with VLDL. Subsequently, isolated LDL was incubated in the presence of bovine milk lipoprotein lipase as a source of triglyceride hydrolysis activity. Lipolysis tended to reduce the size of the major LDL subpopulation, and the mean change in LDL diameter was significantly greater when plasma was preincubated with VLDL supplementation than when it was not \((-0.6 ± 0.3 \text{ versus } -0.2 ± 0.2 \text{ nm, respectively; } P < 0.01)\). Moreover, sequential effects of lipid transfer and lipolysis activities induced dramatic changes in the general shape of gradient gel LDL patterns. The largest plasma LDL subpopulations tended to disappear, and the formation of new, small LDL particles could be observed. The combined effects of neutral lipid transfers and triglyceride hydrolysis could account for variations of gradient gel LDL profiles in human plasma. In particular, these biochemical processes could favor the formation of the small-sized LDL patterns that are associated with an increased risk for coronary artery disease. (Arterioscler Thromb. 1994;14:1327-1336.)

Key Words • LDL • lipid transfers • lipolysis • polyacrylamide gradient gel electrophoresis

Low-density lipoprotein (LDL), the main transport particle for cholesterol in human plasma, has been implicated in the initial events of the formation of atherosclerotic lesions. Plasma LDL is constituted of several discrete subpopulations of distinct size, composition, and metabolic properties. A dichotomous classification of plasma LDL patterns has recently been proposed, on the basis of the gradient gel distribution profiles. Pattern A is defined as an LDL subclass pattern containing mainly large particles (apparent diameter greater than 25.5 nm) with minor subpopulations of smaller size; pattern B is constituted mainly of small LDL particles (mean apparent diameter less than 25.5 nm) with minor subpopulations of larger size. The LDL subclass pattern B, which is significantly associated with an increased risk for coronary artery disease, may relate to variation in a specific genetic locus. However, recent data indicate that LDL particle size would be more greatly influenced by nongenetic factors, and alterations in LDL structure and composition are mainly dependent on environmental factors such as diet and drug therapy. In fact, changes in LDL size mainly seem to reflect variations in lipid status, and the association between LDL size and coronary artery disease is not independent of other established risk factors, among them LDL and high-density lipoprotein (HDL) cholesterol (HDL-C) levels. LDL from hypertriglyceridemic patients is smaller than that from normal control subjects. More recently, plasma triglyceride concentration was designated as the single most important factor affecting LDL particle size, and variations in plasma triglyceride concentration induce alterations in plasma LDL patterns.

In human plasma, the cholesteryl ester transfer protein (CETP) catalyzes the net mass transfer of cholesteryl esters from LDL and HDL toward very-low-density lipoprotein (VLDL), with the net mass transfer of triglycerides in the opposite direction, from VLDL toward LDL and HDL. Therefore, it appears that VLDL, through the neutral lipid transfer reaction catalyzed by CETP, may play an important role in determining the structure and composition of the two other main plasma lipoprotein fractions, LDL and HDL. The LDL pattern in normolipidemic plasma is dependent on
CETP activity. As observed in vitro, the CETP-mediated size redistribution of plasma LDL particles is characterized mainly by a shift of the small particles toward the largest LDL subpopulation. In contrast, the appearance of newly formed LDL subpopulations of smaller size has not been observed, suggesting that CETP may not be the sole factor responsible for alterations in plasma LDL distribution profiles. Thus, formation of small-LDL patterns in human plasma might also relate to other enzyme activities such as lipolysis, which in vitro reduces the size of isolated lipoprotein particles.

In the present study, the implication of neutral lipid transfers in the formation of small LDL particles, which in vivo are associated with high plasma triglyceride levels, was investigated by incubating total human plasma in the presence of bovine milk lipoprotein lipase (LPL) as a source of triglyceride hydrolysis activity. Analysis of resulting LDL patterns by polyacrylamide gradient gel electrophoresis (PAGGE) suggested that the combined effects of neutral lipid transfers and lipolysis could account for variations in LDL patterns among various plasma samples.

**Methods**

**Subjects**

Fifteen subjects (11 women and 4 men; age range, 24 through 50 years; plasma cholesterol concentrations, 151 to 274 mg/dL; and plasma triglyceride concentrations, 47 to 265 mg/dL) were selected for the study. With the exception of four women who took oral contraceptives, the subjects were not taking medications.

**Blood Samples**

Venous blood from all subjects was collected after a 12-hour overnight fast. Blood samples were drawn into EDTA-containing glass tubes, and plasma was promptly separated by a 5-minute centrifugation at 3000g. Plasma was kept at 4°C, and analyses were started within a few hours after sample collection.

**Lipoprotein Preparation**

Lipoprotein fractions were isolated from total plasma by sequential ultracentrifugation. Densities were adjusted by the addition of KBr and checked with a DMA 3S digital densitometer (Paar). VLDL was isolated as the d<1.006 g/mL fraction with two successive 4-hour runs at 60,000 rpm in a 70-Ti rotor on an L7 ultracentrifuge (Beckman). LDL was isolated as the d=1.019<d<1.063 g/mL fraction in a 65-Ti rotor on an L7 ultracentrifuge (Beckman) with one 6-hour/60,000-rpm run at the lowest density and two 15-hour/40,000-rpm runs at the highest density.

**Electrophoretic Separation of LDL Subfractions**

LDL was separated by electrophoresis on nondenaturing gradient gels ranging from 20 to 160 g/L polyacrylamide (PAA 2/16, Pharmacia). The electrophoresis was performed at 4°C for 26 hours: 2 hours at 30 V, 12 hours at 50 V, and 12 hours at 150 V. At the end of the electrophoresis the gels were fixed, stained with Coomassie brilliant blue G, and destained. 30 The distribution profile of lipoprotein subfractions was finally obtained by densitometric scanning of the gels at 633 nm with a 2202 Ultrascan laser densitometer (LKB) attached to a 2220 integrator (LKB) that directly indicated the position of the predominant LDL peak.

The apparent diameters of the separated LDL subfractions were determined by comparison with ferritin (diameter, 12.20 nm), thyroglobulin (diameter, 17.00 nm; Pharmacia High Molecular Weight Protein Calibration kit), and carboxylated latex beads (diameter, 38.00 nm; Duke Scientific) that were subjected to electrophoresis with the samples. The reproducibility of the gel analysis of LDL size was evaluated by analyzing the same LDL preparation eight times on the same gel and by running eight different gels independently. Standard deviations of mean LDL diameter (26.50 nm) within the same gel and between runs were 0.06 and 0.19 nm, respectively. Throughout the study different experimental conditions applied to the same plasma sample were analyzed on the same gel.

**Enrichment of LDL With Triglycerides**

Human LDL was progressively enriched with triglycerides by supplementing total plasma with various amounts of ultracentrifugally isolated VLDL. The final triglyceride concentration of added VLDL ranged from 0 to 2.0 g/L. Subsequently, mixtures were incubated for 24 hours at 37°C to allow the substitution of triglycerides for cholesteryl esters in LDL particles through the CETP-mediated neutral lipid transfer reaction (see "Results"). To block lecithin:cholesterol acyltransferase activity, all plasma samples contained 1.5 mmol/L iodoacetate.

**Lipoprotein Lipolysis**

Isolated LDL was lipolysed by incubation in the presence of purified bovine milk LPL (specific activity, 4000 U/mg) (Sigma). Briefly, LDL (protein concentration, 0.5 mg/mL), LPL (concentration range, 0.34 to 6.20 μg/mL), heparin (2 U/mL), and fatty acid–poor bovine albumin (60 mg/mL) were incubated at 37°C in a buffer of 0.2 mol/L tris(hydroxymethyl)aminomethane and 0.08 mol/L NaCl, pH 8.4 (total incubation volume, 1 mL). At the end of the incubation LPL activity was inhibited by the addition to each tube of 0.33 g KBr, which raised the final density of the incubation mixtures to d=1.21 g/mL. LDL particles were then reisolated by one 15-hour ultracentrifugation at 40,000 rpm on a 50.4-Ti rotor on an L7 ultracentrifuge (Beckman).

**Lipoprotein Component Assays**

All chemical assays were performed on a Cobas-Fara centrifugal analyzer (Roche). Protein concentrations were measured by using biocinchonic acid reagent (Pierce) according to the method of Smith et al. Total cholesterol, unesterified cholesterol, phospholipid, and triglyceride concentrations were measured by enzymatic methods using Boehringer reagents. Plasma HDL-C concentrations were measured after selective precipitation of apolipoprotein (apo) B–containing lipoproteins with Boehringer phosphotungstic acid/MgCl2 reagent as recommended by the manufacturer. Plasma VLDL + LDL cholesterol concentrations were obtained by difference.

**Statistical Evaluation**

ANOVA was used to determine the significance of the difference between data means. Coefficients of correlation, r and p, were calculated by using linear regression and Spearman rank correlation analysis, respectively. Multiple regression analysis was used to determine significant contributions of HDL-C and triglycerides to the prediction of LDL size.

**Results**

**Baseline Characteristics of Study Subjects**

Table 1 presents the lipoprotein parameters of the various plasma samples used throughout this study. The mean apparent diameter of the major plasma LDL subfraction as determined by PAGGE correlated nega-
tively with plasma triglyceride concentrations and positively with HDL-C levels (Table 2). In addition, a significant positive correlation between LDL size and HDL/VLDL+LDL cholesterol ratios was observed. Multiple regression analysis24 revealed further that plasma triglyceride and HDL-C concentrations, when combined in a two-variable model, accounted for 79% of the variability in LDL size, with only triglycerides reaching a significant level in the present study (P<.02). Subsequent experiments were designed to investigate the mechanism that may account in human plasma for the relationships between LDL size and plasma lipid parameters.

Correlation of Plasma LDL Size With Neutral Lipid Transfers
To determine whether plasma LDL size could relate to its ability to exchange cholesteryl esters for triglycerides, the apparent diameter of the major LDL subtraction in native plasma samples was compared with changes in the LDL cholesteryl ester/protein ratio and the LDL triglyceride/protein ratio induced by the incubation of total plasmas for 24 hours at 37°C.

LDL size correlated positively with changes in the LDL cholesteryl ester/protein ratio (r=.79, P<.001 using regression analysis; ρ=.60, P<.03 using Spearman rank correlation analysis) and negatively with changes in the LDL triglyceride/protein ratio (r=−.72, P<.005 using regression analysis; ρ=−.44, P=NS using Spearman rank correlation analysis) (Fig 1). These observations suggested that an elevated capacity of plasma LDL to acquire triglycerides and to donate cholesteryl esters would be associated with a preponderance of small-LDL subpopulations in human plasma.

Effect of Lipid Transfers Between VLDL and LDL on the Size and Composition of LDL Particles
To investigate the implication of lipid transfers between triglyceride-rich lipoproteins and LDL in determining plasma LDL pattern, total human plasma containing a lecithin:cholesterol acyltransferase inhibitor (iodoacetate 1.5 mmol/L) was incubated for 24 hours at 37°C in the absence or presence of various concentrations of VLDL (added triglyceride concentrations ranged from 0.5 to 2.0 g/L). At the end of the incubation, plasma LDL patterns were determined by gradient gel electrophoresis of the 1.019<rf<1.063 g/mL plasma lipoprotein fraction.

Incubation of total plasma tended to induce the transformation of polydisperse patterns into monodisperse profiles26 (Fig 2). This phenomenon was characterized by a decrease in the relative abundance of discrete, minor LDL subpopulations. When plasma was supplemented with increasing concentrations of VLDL before incubation for 24 hours at 37°C, the general shape of the LDL

distribution profile was not affected further (Fig 2). However, with the highest VLDL concentration studied (added triglyceride concentration, 2.0 g/L) a slight but significant increase in the mean size of the major LDL peak was observed compared with the peaks observed in both 4°C control plasma and incubated plasma without VLDL supplementation (Table 3).

Changes in LDL size were accompanied by alterations in composition of the particles. Incubation of total plasma induced a significant decrease of the unesterified cholesterol/protein and cholesteryl ester/protein ratios in LDL particles with a concomitant increase in the triglyceride/protein ratio. These changes in LDL composition were greater when total plasma was supplemented with VLDL (added triglyceride concentration, 2.0 g/L) (Table 3). No significant differences in the mean size of the major LDL subpopulation between the Control and Transfer groups were observed, suggesting that changes in lipid composition of LDL particles as induced by plasma incubation without VLDL supplementation were not high enough to induce detectable variations in LDL diameter (Table 3).

Significant relationships between changes in size and the lipid/protein ratio in LDL particles were observed. Indeed, changes in LDL size correlated positively with changes in the triglyceride/protein ratio but negatively with changes in the cholesteryl ester/protein ratio ($r = -0.67, P = .0001$ and $r = -0.55, P = .0015$, respectively). By contrast, no significant correlations were observed with changes in either the unesterified cholesterol/protein, phospholipid/protein, or total lipid/protein ratios (Fig 3).

Whereas the results described above indicate that the replacement of cholesteryl esters by triglycerides in the LDL core may be an important process in determining the size and distribution of plasma LDL, they alone cannot account for the negative correlation between the size of the major LDL subfraction and plasma triglyceride levels that has been observed in population studies.

### Concentration-Dependent Effect of LPL on LDL Patterns

To investigate the implication of triglyceride hydrolysis in changing plasma LDL patterns, the concentration-dependent effect of bovine milk LPL on the size distribution of either native or triglyceride-enriched LDL particles was studied. To this end, LDL particles were isolated from

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**Table 3. Effect of Lipid Transfers on the Size and Composition of Plasma LDL Particles**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Transfer</th>
<th>Transfer+VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size major LDL</td>
<td>25.3±0.7</td>
<td>25.2±0.7</td>
<td>25.5±0.6†</td>
</tr>
<tr>
<td>UC/protein ratio</td>
<td>0.62±0.12</td>
<td>0.56±0.10†</td>
<td>0.52±0.08‡§</td>
</tr>
<tr>
<td>CE/protein ratio</td>
<td>1.94±0.67</td>
<td>1.75±0.55§</td>
<td>1.37±0.42‡§</td>
</tr>
<tr>
<td>PL/protein ratio</td>
<td>1.01±0.29</td>
<td>0.97±0.24</td>
<td>1.00±0.21</td>
</tr>
<tr>
<td>TG/protein ratio</td>
<td>0.29±0.05</td>
<td>0.50±0.24†</td>
<td>1.01±0.26‡§</td>
</tr>
</tbody>
</table>

Aliquots from 15 plasma samples (see Table 1) were supplemented with a lecithin:cholesterol acyltransferase inhibitor (iodoacetate, 1.5 mmol/L) and were maintained at 4°C (Control) or incubated for 24 hours at 37°C without (Transfer) or with (Transfer+VLDL) VLDL supplementation (final concentration of added triglycerides, 2.0 g/L). Subsequently, LDL was isolated by ultracentrifugation and analyzed (see "Methods"). LDL indicates low-density lipoprotein; VLDL, very-low-density lipoprotein; UC, unesterified cholesterol; CE, cholesteryl ester; PL, phospholipid; and TG, triglyceride.

*P<.05, †P<.001, ‡P<.0001 vs Control. §P<.05, §P<.01, ‡P<.0001 vs Transfer.
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plasma samples that were (Transfer+VLDL samples) or were not (Control samples) preincubated in the presence of VLDL (added triglyceride concentration, 2.0 g/L). Isolated LDL was then incubated for 1 hour at 37°C in the presence of increasing concentrations of bovine milk LPL as a source of triglyceride hydrolysis activity. LPL did not induce marked changes in the size distribution of native LDL that was isolated from normolipidemic plasma (Fig 4). In contrast, when LDL was isolated from plasma preincubated in the presence of VLDL, LPL induced a concentration-dependent shift of the gradient gel distribution profile of triglyceride-enriched LDL toward subpopulations of a smaller size (Fig 4). Thus, the LPL-mediated reduction of LDL size would require as a first step the substitution of triglycerides for cholesteryl esters through neutral lipid transfer reactions.

Combined Effects of Neutral Lipid Transfers and Lipolysis on LDL Patterns

To further investigate the possible interaction of neutral lipid transfers and lipolysis in the transformation of plasma LDL patterns, total plasma with or without VLDL (added triglyceride concentration, 2.0 g/L) was preincubated for 24 hours at 37°C. Subsequently, isolated LDL was incubated for 2 hours at 37°C in the presence of bovine milk LPL (final concentration, 6.2 μg/mL) as a source of triglyceride hydrolysis activity. Finally, the mean apparent diameter of the major LDL subpopulation was determined by PAGE of the LDL fraction.

With native LDL (Control samples), the sole effect (if any) of LPL was characterized by a slight decrease in the mean apparent diameter of the major LDL subpopulation. Indeed, in eight plasma samples studied, the mean variation in lipolyzed compared with nonlipolyzed samples \( \Delta\bar{D}_{LPL} - \Delta D_{+LPL} \) was a 0.2-nm reduction in the mean apparent diameter of LDL (Table 4). When LDL was obtained from preincubated plasma samples without VLDL supplementation (Transfer samples), LPL decreased the LDL diameter in all samples studied; in those instances, the mean reduction of LDL size was nonsignificantly greater compared with control samples (0.45 versus 0.20 nm, respectively) (Table 4). When plasma samples were preincubated in the presence of VLDL (Transfer+VLDL samples), LPL induced a significantly greater reduction in LDL size compared with Control homologues (−0.60 versus −0.20 nm, respectively; \( P<.01 \)) (Table 4).
TABLE 4. Effect of Lipolysis and Lipid Transfers on the Size of the Major LDL Subpopulation

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control −LPL</th>
<th>+LPL</th>
<th>Δ (−LPL) (+LPL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>25.2</td>
<td>25.2</td>
<td>0.0</td>
</tr>
<tr>
<td>9</td>
<td>25.3</td>
<td>25.3</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>25.9</td>
<td>25.4</td>
<td>−0.5</td>
</tr>
<tr>
<td>11</td>
<td>26.0</td>
<td>25.8</td>
<td>−0.2</td>
</tr>
<tr>
<td>12</td>
<td>23.6</td>
<td>23.3</td>
<td>−0.3</td>
</tr>
<tr>
<td>13</td>
<td>25.8</td>
<td>25.6</td>
<td>−0.2</td>
</tr>
<tr>
<td>14</td>
<td>23.9</td>
<td>23.5</td>
<td>−0.4</td>
</tr>
<tr>
<td>15</td>
<td>25.6</td>
<td>25.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>25.2±0.9</td>
<td>25.0±1.0</td>
<td>−0.2±0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transfer −LPL</th>
<th>+LPL</th>
<th>Δ (−LPL) (+LPL)</th>
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<tbody>
<tr>
<td>8</td>
<td>25.3</td>
<td>25.2</td>
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<tr>
<td>9</td>
<td>25.4</td>
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<td>10</td>
<td>25.8</td>
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<td>15</td>
<td>25.9</td>
<td>25.2</td>
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<tr>
<td>Mean±SD</td>
<td>25.2±0.8</td>
<td>24.8±0.9</td>
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<table>
<thead>
<tr>
<th>Transfer+VLDL −LPL</th>
<th>+LPL</th>
<th>Δ (−LPL) (+LPL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>25.7</td>
<td>25.2</td>
</tr>
<tr>
<td>9</td>
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<td>23.6</td>
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<tr>
<td>15</td>
<td>26.3</td>
<td>25.2</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>25.5±0.7</td>
<td>24.8±0.8</td>
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</table>

Whereas the combined effects of lipid transfers and lipolysis induced consistent changes among the various plasma samples studied, some variations could be observed according to the starting plasma LDL pattern. Fig 5 represents two series of LDL profiles obtained either from a normolipidemic plasma that initially showed a typical LDL pattern A (subject 11) or from a moderately hypertriglyceridemic plasma that initially showed a typical LDL pattern B (subject 14). Whereas in vitro lipolysis of LDL isolated from nonincubated plasma (Control + LPL profiles) reduced only slightly the mean apparent diameter of the major but not the minor LDL subpopulation of pattern A, it more dramatically reduced the apparent diameter of each of the three LDL subpopulations of pattern B. However, in both cases, the general shape of LDL distribution profiles remained virtually the same.

Incubation of total plasma from subject 11 (Transfer profile) induced only minor alterations in LDL pattern A; the smallest LDL subpopulation (24.4 nm diameter) tended to shift toward the largest one (Fig 5). More pronounced transformations were observed in LDL pattern B, since incubation of total plasma from subject 14 (Transfer profile) induced the redistribu-
obtained under similar experimental conditions, the cause of the somewhat different effects of plasma incubation on the LDL phospholipid/protein ratio is unclear and might relate to interindividual variations in the exchangeable phospholipid pool, a parameter that we did not investigate. Incubation of LDL in the presence of LPL was characterized mainly by a significant reduction of the triglyceride/protein ratio, whereas the unesterified cholesterol and cholesteryl ester contents remained unchanged (Table 5). LPL also slightly reduced the phospholipid content of LDL particles compared with nonlipolyzed homologues (Table 5). The ability of LPL to hydrolyze LDL triglycerides appeared to be dependent on the triglyceride content of LDL particles. Indeed, compared with nonlipolyzed homologues, the mean reduction of the LDL triglyceride/protein ratio was 0.06, 0.27, and 0.48 in LDL isolated from Control, Transfer, and Transfer+VLDL plasma samples, respectively.

**Discussion**

Results from the present study revealed that in human plasma, both lipid transfer and triglyceride hydrolysis may constitute two key events in the transformation of gradient gel LDL patterns. Whereas lipid transfer activity or lipolysis alone promoted only minor changes in the mean size of the major plasma LDL subpopulation, their combined effects induced the formation of small LDL particles. The resulting LDL patterns presented the main features of the LDL pattern B, which is associated with an increased risk for coronary artery disease.12

Earlier in vitro studies, Deckelbaum and coworkers27 proposed a general model for the size reduction of isolated LDL particles involving triglyceride hydrolysis in addition to neutral lipid transfers. Moreover, recent data indicate that the presence of small LDL particles in human plasma could be related to the efficiency of the lipolytic system.1437 In vitro changes in the size of LDL particles as induced during the incubation of isolated...
lipoprotein fractions with a crude source of lipid transfer activity and purified LPL have been investigated by using electron microscopy and rate zonal ultracentrifugation. Since these two techniques do not allow the investigation of the specific aspects of plasma LDL heterogeneity that can be observed by using PGGE, in the present study we investigated the combined effects of lipid transfers and lipolysis on the gradient gel electrophoretic patterns of the plasma LDL fraction.

The mean apparent diameter of the major LDL subfraction in human plasma in the population studied, as determined by laser densitometric scanning of polyacrylamide gradient gels, correlated negatively with triglyceride levels but positively with HDL-C levels. These observations agree with previous data that show that the abundance of small LDL particles correlates positively with plasma triglyceride, total cholesterol, VLDL cholesterol, and apoB concentrations but negatively with plasma HDL-C and apoA-I levels. In addition, the preponderance of small LDL particles in human plasma appears to be significantly associated with an increased risk for coronary artery disease. However, adjustment for triglyceride concentration reduces the risk ratio of the small-LDL pattern (pattern B) to a nonsignificant level. In fact, the triglyceride content of LDL particles is a predictable function of plasma triglyceride concentration, and the amount of circulating triglycerides is the single most important factor affecting LDL particle size.

Whereas a strong, negative correlation between plasma triglyceride levels and LDL size clearly exists, the biochemical mechanism by which triglycerides can affect the gradient gel pattern of the plasma LDL fraction is still not completely understood. Interestingly, in the present study the diameter of the major plasma LDL subfraction correlated positively with the plasma HDL/VLDL+LDL cholesterol ratio, which correlates negatively with plasma CETP activity. These observations suggest, therefore, that CETP-mediated neutral lipid transfers could play a determinant role not only in determining the polydispersity or monodispersity of plasma LDL pattern but also in modifying the mean size of the major LDL subclass in human plasma. This hypothesis was sustained by another finding of the present study, which revealed that the size of the major plasma LDL subfraction correlates negatively with the ability of LDL particles to acquire triglyceride and to donate cholesterol esters during the incubation of total plasma. In human plasma, VLDL concentrations can influence the CETP-mediated lipid transfer process; Mann and coworkers report a threefold increase in lipid transfers between plasma LDL+HDL and VLDL fractions in hypertriglyceridemic subjects compared with normolipidemic control subjects. In hypertriglyceridemia, increased neutral lipid exchanges can result in the replacement of cholesterol ester by triglyceride as the predominant neutral lipid component in the LDL core. When circulating triglyceride levels are lowered, the composition of hypertriglyceridemic LDL can be normalized as the result of a reduction of the rate of transfer of neutral lipids between the VLDL and LDL+HDL fractions.

To study the implication of lipid transfers in modulating the size distribution of plasma LDL particles, total human plasma was incubated for 24 hours at 37°C. In a parallel series of experiments, plasma was supplemented with VLDL particles, which served both as a donor for triglycerides to LDL and as an acceptor for cholesterol esters that leave LDL. We observed that incubation of total human plasma induced alterations of both the distribution profile and composition of plasma LDL particles. However, lipid transfer reactions, which promoted enrichment of the LDL core with triglycerides, did not promote the formation of the small-LDL subpopulations that have been observed in hypertriglyceridemic states. On the contrary, replacement of cholesterol esters by triglycerides in LDL particles tended to promote the enlargement of LDL particles, which probably reflected the greater volume of triglyceride molecules compared with cholesteryl ester molecules. Therefore, these data indicated that the association of small LDL particles with elevated plasma triglyceride concentrations would involve more than lipid transfer reactions and suggested that an additional event was required to allow the shift of large LDL toward particles of smaller size.

In postheparin plasma, two distinct triglyceride lipases have been identified: LPL, which originates from extrahepatic tissues, and hepatic lipase (HL), which originates from the liver. Whereas both lipases are able to interact with various plasma lipoprotein fractions, HL has an increasing activity with lipoproteins of decreasing size, and VLDL appears to be a better substrate than LDL for LPL. However, despite differences in the substrate specificity of LPL and HL, in vitro studies reveal that the two enzymes induce very similar alterations in LDL composition that are characterized mainly by a substantial reduction in the core triglyceride content. In the present study, we chose to hydrolyze triglycerides in LDL particles by using a commercially available preparation of purified bovine milk LPL that has structural and functional similarities to human LPL. When plasma LDL was incubated with LPL, the hydrolysis of core triglycerides was accompanied by a concomitant reduction of the size of the major LDL subfraction. This observation was consistent with previous data that demonstrate that the removal of triglycerides from the large, triglyceride-rich LDL subfractions as mediated by either LPL or HL induces their conversion toward LDL subpopulations of smaller size. In the present study the LPL-mediated reduction of LDL size may be accompanied by a small decrease in the LDL phospholipid/protein ratio, which supports the observation by Barter and coworkers that the molar phospholipid/apoB ratio is an important parameter in determining the size of LDL particles. The sequential effects of lipid transfer and lipolysis activities promoted dramatic changes not only in the mean size of plasma LDL but also in the general shape of their gradient gel distribution profiles. In particular, when the effects of lipid transfers and lipolysis were combined, the conversion of LDL pattern A into LDL pattern B could be observed. Therefore, we postulate that in vivo, CETP and triglyceride lipases could have synergistic effects in favoring the formation of the potentially atherogenic LDL pattern B. Plasma neutral lipid transfers, which are dependent on plasma VLDL concentrations rather than on CETP mass, might constitute a limiting step in the transformation of the plasma LDL pattern, since in our experiments LPL did not induce marked changes in the distri-
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References


Combined effects of lipid transfers and lipolysis on gradient gel patterns of human plasma LDL.

L Lagrost, P Gambert and C Lallemant

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