Determinants of LDL Subfraction Distribution and Concentrations in Young Normolipidemic Subjects

T.D.G. Watson, M.J. Caslake, D.J. Freeman, B.A. Griffin, J. Hinnie, C.J. Packard, J. Shepherd

Abstract Human low-density lipoproteins (LDLs) comprise a spectrum of particles that vary in size, density, chemical composition, metabolic behavior, and atherogenicity. To identify determinants of this heterogeneity, we measured the percent distribution and plasma concentrations of the three major LDL subfractions in 34 young healthy subjects. These parameters were correlated in univariate and multivariate analyses with various body and lifestyle factors; plasma lipids and lipoproteins; and the activities of cholesteryl ester transfer protein, lipoprotein lipase, and hepatic lipase (HL). Women (n=15) had significantly more large, buoyant LDL (LDL-I; density, 1.025 to 1.034 g/mL) and high-density lipoprotein (HDL-I) than men (n=19). Both the percentage and concentration of LDL-I were correlated negatively with very-low-density lipoprotein triglycerides (VLDL-TG) and HDL; they were correlated positively with HDL-cholesterol (HDL-C) and HDL-I. In addition, percent LDL-I was negatively correlated with plasma triglycerides, VLDL-C, LDL-C, and apoB concentrations. The concentrations of intermediate and small, dense LDL (LDL-II and LDL-III; density, 1.034 to 1.044 and 1.044 to 1.060 g/L, respectively) were positively correlated with LDL-C. LDL-III concentrations were also related to plasma cholesterol and apoB concentrations and HL activity. On multivariate analyses, approximately one third of the variability in LDL-I was explained by HL and plasma triglycerides. More than 80% of the variation in LDL-II was accounted for by a model that combined LDL-C and plasma apoB with body mass index and VLDL-TG. Plasma apoB concentrations also featured in multivariate models of LDL-III and together with HL and lipoprotein(a) explained approximately one third of its variability. The activity of HL accounted for the differences in LDL-I between men and women, the reciprocal relation between LDL-I and LDL-III, and the close association of LDL-I with HDL-I. These data confirm that HL is a major determinant of LDL subfraction distribution and indicate, through coordinate regulation of LDL-I and HDL-I, that these two subfractions should not be considered as independent predictors of coronary risk. (Arterioscler Thromb. 1994;14:902-910.)

Key Words • LDL subfractions • HDL subfractions • lipoprotein lipase • hepatic lipase • cholesteryl ester transfer protein

It is now clear that plasma low-density lipoproteins (LDLs), traditionally considered a homogeneous entity, actually comprise a spectrum of particles that vary in size, density, and chemical composition (reviewed in Reference 1). Furthermore, it has been shown that LDL subpopulations may differ in their metabolic behavior and atherogenic potential. For instance, a preponderance of small, dense LDL has been associated with an increased risk of myocardial infarction and both the presence and severity of coronary artery disease. This pattern has been implicated, along with raised plasma triglycerides and low levels of high-density lipoproteins (HDLs), as a potent atherogenic lipoprotein phenotype. The origins of LDL heterogeneity have been examined in a small number of cross-sectional studies. These have identified age, sex, plasma lipid and lipoprotein levels, the magnitude of postprandial lipemia, and a genetic trait as possible determinants of LDL subfraction distribution. Other influences may include adiposity and exercise, hormonal status, and pharmacological agents that affect lipid and lipoprotein metabolism.

Men are more likely than women to have small, dense LDLs, whereas levels of large, buoyant LDL are higher in women, and it has been suggested that this might be mediated through male-female differences in the activity of hepatic lipase (HL). It has also been suggested that lipoprotein lipase (LPL) and cholesteryl ester transfer protein (CETP), which is capable of inducing size changes in LDL in vitro, could remodel LDL and therefore regulate its subfraction distribution. A number of studies have identified a link between plasma triglycerides and LDL subfraction pattern, in particular between raised triglycerides and small, dense LDL. The mechanism underlying this is thought to involve neutral lipid exchange, whereby triglycerides are transferred into LDL from triglyceride-rich lipoproteins in exchange for cholesteryl esters. This may then render the LDL susceptible to the action of endothelial lipases with resultant reduction in particle size and the generation of small, dense LDL.

The aim of the present study was to identify possible determinants of LDL heterogeneity in a group of young, healthy, nonobese normolipidemic men and women. Three major LDL subfractions were isolated by density gradient centrifugation and their distribution and plasma concentrations correlated with a number of body and lifestyle factors; plasma lipid and lipoprotein concentrations; and the activities of LPL, HL, and CETP. This study was specifically designed to examine how the
distribution of LDL subfractions is regulated in people with a preponderance of larger LDL species corresponding to the normal pattern (pattern A) described by Krauss and Blanche. We also addressed the possibility that metabolic relations may exist between LDL and HDL subfractions, as suggested by a previous longitudinal study in healthy men.24

Methods

Subjects and Samples

Thirty-four healthy volunteers (19 men, 15 women) aged 19 to 49 years were recruited from the staff at the Royal Infirmary, Glasgow. All of the subjects were normotriglyceridemic (plasma triglycerides ≤2.1 mmol/L), normotensive, nonobese, and had a weekly alcohol consumption of less than 21 units. Plasma cholesterol concentrations were less than 6.5 mmol/L in 32 of the subjects; the exceptions were two male subjects with cholesterol concentrations of 6.7 and 6.8 mmol/L who were retained in the study because of their normal triglycerides. The study was approved by the Research Ethics Committee of the Glasgow Royal Infirmary; each subject gave written informed consent.

All volunteers were sampled after an overnight fast of 12 hours. Blood (50 mL) was collected by venipuncture and placed in K2 EDTA (final concentration, 1 mg/mL). Plasma was harvested at 4°C by low-speed centrifugation; aliquots for lipid and lipoprotein measurements and fractionation were used immediately, and those for CETP and lipoprotein (a) (Lp[a]) were frozen at -20°C. Postheparin plasma was then obtained from blood collected 10 minutes after intravenous injection of 70 U heparin per kilogram body weight. The samples were placed on ice; the plasma was separated as above and stored immediately at -70°C.

Plasma Lipids and Lipoproteins

Cholesterol and triglyceride concentrations were measured using commercially available kits (816302 and 816370; BCL). Lipoprotein-cholesterol concentrations were determined after isolation of very-low-density lipoprotein (VLDL) (in which triglyceride concentrations were also measured) by ultracentrifugation and precipitation of LDL from HDL in the infranatant by heparin-manganese chloride and the percent transfer of [3H]cholesterol oleate was measured by liquid scintillation. The specific activity of CETP was expressed as percent transfer per 2.5 hours of incubation per microliter of plasma, and the interassay coefficient of variation was less than 10%.

Lipoprotein Lipase and Hepatic Lipase

The activities of LPL and HL were measured in postheparin plasma incubated with a gum arabic-stabilized triglyceride emulsion containing glycerol tri[1-14C]oleate (Amersham International plc) at a specific activity of 30 μCi/mmol triglyceride fatty acids. The fatty acids released by lipolysis were captured by bovine serum albumin and extracted by solvent partitioning for counting by liquid scintillation. LPL was measured in postheparin plasma that had been preincubated with sodium dodecyl sulfate to inhibit HL, and the incubation included pooled plasma as a source of apoC-II for the activation of LPL. In the assay of HL, the incubation mixture contained 1.0 mol/L NaCl to inactivate LPL, and the serum was excluded. Enzyme activities are expressed in micromoles of fatty acids released per hour per milliliter of plasma; the intra-assay and interassay coefficients of variation for the two enzyme assays have been reported elsewhere.

Statistical Methods

Statistical manipulations and significance testing were performed using the personal computer version of MINTAB RELEASE 8 (Minitab Inc). Two group comparisons were made using the Mann-Whitney U test for nonparametric data. The distribution of each variable was assessed by drawing normality plots; the following were not normally distributed and were transformed as shown in parentheses: plasma and VLDL triglycerides (log10), HDL-C (log10), Lp(a) (log10), apoA-I (log10), HDL-2 (square root), percentage and mass of LDL-I and HDL-II, and percentage and mass of LDL-III (log10).

Results

Body and Lifestyle Factors, Plasma Lipids, Lipoproteins, and Intravascular Enzymes

There were no differences in age, body mass index (BMI), and number of smokers in the male and female groups in the study (Table 1). Systolic and diastolic blood pressures were significantly higher in men. Cho-
TABLE 1. Characteristics of the Study Group: Body Factors, Plasma Lipids and Lipoproteins, Cholesteryl Ester Transfer Protein, and Postheparin Lipase Activities

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group (n=34)</th>
<th>Men (n=19)</th>
<th>Women (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>31.7 (7.8)</td>
<td>31.4 (7.8)</td>
<td>32.1 (8.0)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.8 (2.4)</td>
<td>23.9 (2.4)</td>
<td>23.6 (2.4)</td>
</tr>
<tr>
<td>No. of smokers</td>
<td>11</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>118 (13)</td>
<td>123 (13)</td>
<td>114 (11)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>75 (10)</td>
<td>78 (9)</td>
<td>72 (9)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.93 (0.86)</td>
<td>5.05 (1.03)</td>
<td>4.78 (0.59)</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>0.96 (0.36)</td>
<td>1.06 (0.41)</td>
<td>0.84 (0.23)</td>
</tr>
<tr>
<td>VLDL-C, mmol/L</td>
<td>0.49 (0.15)</td>
<td>0.55 (0.15)</td>
<td>0.43 (0.11)</td>
</tr>
<tr>
<td>VLDL-TG, mmol/L</td>
<td>0.49 (0.30)</td>
<td>0.61 (0.35)</td>
<td>0.36 (0.14)</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.09 (0.85)</td>
<td>3.30 (0.95)</td>
<td>2.81 (0.65)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.35 (0.32)</td>
<td>1.21 (0.27)</td>
<td>1.54 (0.28)</td>
</tr>
<tr>
<td>ApoA-I, mg/dL</td>
<td>1.19 (0.17)</td>
<td>1.13 (0.15)</td>
<td>1.23 (0.17)</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>0.83 (0.25)</td>
<td>0.91 (0.28)</td>
<td>0.73 (0.17)</td>
</tr>
<tr>
<td>Lipoprotein(a), mg/dL</td>
<td>7.0 (1-88)</td>
<td>7.0 (1-11)</td>
<td>9.0 (1-88)</td>
</tr>
<tr>
<td>Lipoprotein lipase*</td>
<td>5.82 (2.48)</td>
<td>5.58 (1.90)</td>
<td>6.12 (3.31)</td>
</tr>
<tr>
<td>Hepatic lipase*</td>
<td>11.96 (4.81)</td>
<td>13.56 (4.35)</td>
<td>9.94 (5.60)</td>
</tr>
<tr>
<td>CETP†</td>
<td>45.5 (14.5)</td>
<td>47.7 (16.2)</td>
<td>43.3 (15.6)</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; VLDL-C, very-low-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Apo, apolipoprotein; and CETP, cholesteryl ester transfer protein. Values are mean (SD) with the exception of lipoprotein(a), where the median and range are given.

*Expressed in micromoles of fatty acids released per hour per milliliter of plasma.
†Expressed as percent transfer per 2.5 hours of incubation per microliter of plasma.

*P<.05, §P<.01, ||P<.005, men vs women, by Mann-Whitney test.

Lesterol, triglyceride, VLDL triglyceride (VLDL-TG), LDL cholesterol (LDL-C), and Lp(a) concentrations did not differ, but VLDL-C concentrations were significantly higher in men, and HDL-C and apoA-I were higher in women. Three apoE phenotypes were represented in the male group (five 3/2, eight 3/3, and six 4/3), and the women demonstrated five (one 2/2, three 3/2, six 3/3, two 4/2, and three 4/3). There were no significant differences in the activities of CETP and LPL between men and women, whereas HL was significantly lower in the women, with the median and range being 73% of that of the men.

LDL and HDL Subfraction Distributions

LDL-II was the major LDL subfraction in 28 of the 34 subjects; LDL-I predominated in three women, and in three men LDL-III was the major species. The percentage and plasma concentration of LDL-I and the plasma concentrations of HDL₃ were significantly higher in women, who had approximately twice as much of these lipoprotein subfractions as their male counterparts (Table 2, Fig 1). There were no significant intersex differences in the percentage and concentration of LDL-II and the concentration of HDL₁. Although the percentage and concentration of LDL-III parameters were higher in men, the differences were not statistically significant. The large standard deviations associated with the male LDL-III data were attributable to two individuals who had LDL-III percentages and concentrations in excess of 60% and 300 mg/dL, respectively, while in the other subjects these were less than 30% and 100 mg/dL, respectively.

In the study group as a whole there were significant interrelations between percent LDL-I and percent LDL-III (r=-.63, P<.0005), the concentrations of LDL-I and LDL-III (r=-.53, P<.0005), and percent LDL-II and percent LDL-III (r=-.57, P<.0005).

TABLE 2. Percent Distribution of LDL Subfractions and Plasma Concentrations of LDL and HDL Subfractions in the Study Group and Male and Female Subgroups

<table>
<thead>
<tr>
<th>Subfraction</th>
<th>Group (n=34)</th>
<th>Men (n=19)</th>
<th>Women (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-I</td>
<td>21.6 (13.4)</td>
<td>14.8 (9.1)</td>
<td>30.9t (12.9)</td>
</tr>
<tr>
<td>LDL-II</td>
<td>60.2 (16.8)</td>
<td>62.7 (19.4)</td>
<td>56.7 (12.4)</td>
</tr>
<tr>
<td>LDL-III</td>
<td>18.3 (18.5)</td>
<td>22.6 (22.9)</td>
<td>12.4 (7.5)</td>
</tr>
<tr>
<td>Plasma concentration, mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-I</td>
<td>61.4 (38.8)</td>
<td>43.8 (25.0)</td>
<td>85.3* (44.2)</td>
</tr>
<tr>
<td>LDL-II</td>
<td>180.2 (69.3)</td>
<td>194.4 (76.3)</td>
<td>161.0 (55.5)</td>
</tr>
<tr>
<td>LDL-III</td>
<td>59.4 (77.7)</td>
<td>78.5 (98.2)</td>
<td>33.5 (16.2)</td>
</tr>
<tr>
<td>HDL-2</td>
<td>66.6 (39.1)</td>
<td>45.7 (26.2)</td>
<td>93.0† (37.3)</td>
</tr>
<tr>
<td>HDL-3</td>
<td>242.3 (56.8)</td>
<td>232.4 (64.9)</td>
<td>254.7 (43.4)</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; HDL, high-density lipoprotein. Values are mean (SD).
*P<.001, †P<.0005, men vs women, by Mann-Whitney test.
Relations Between LDL Composition and Subfraction Distribution and Body and Lifestyle Factors, Plasma Lipids, Lipoproteins, and Associated Enzymes

On simple univariate analysis, the total plasma concentration of LDL (in milligrams per deciliter) was associated significantly with age \( (r=.62, P<.0005) \) but not with any other body and lifestyle factors. Total plasma LDL concentration was also correlated positively with plasma cholesterol, triglyceride, VLDL-TG, LDL-C, and apoB concentrations and the activity of HL (Fig 2), whereas it was negatively correlated with HDL \( _2 \) (Table 3). The composition of LDL was not related to any of the body and lifestyle factors or plasma lipid and lipoprotein variables. The triglyceride content of LDL was, however, inversely related to HL activity \( (r=-.55, P<.005) \).

Neither the percent distribution nor plasma concentration of LDL subfractions was correlated with any of the body and lifestyle factors. The percentage of LDL-I was inversely related to plasma triglycerides, VLDL-C, VLDL-TG, LDL-C, and apoB in the study group as a whole (Table 3). The association with plasma triglycerides was significant in women \( (r=-.54, P<.05) \) but not in men. The correlations between percent LDL-I and VLDL-C and VLDL-TG were not significant in either the male or female subgroups. The relation between percent LDL-I and LDL-C and apoB was present in men only \( (r=-.51, P<.05 \text{ and } r=-.6, P<.01, \text{ respectively}) \). The plasma concentration of LDL-I was also inversely related to VLDL-TG, but as with percent LDL-I this was not significant in either the male or female subgroups. Both the percentage and concentration of LDL-I were correlated positively with HDL-C and HDL \( _2 \) concentrations and negatively with HL activity in the whole study group. These relations were significant in men only when the percentage and concentration of LDL-I were correlated with HDL-C \( (r=.73, P<.0005 \text{ and } r=.67, P<.005, \text{ respectively}) \), HDL \( _2 \) \( (r=.67, P<.005 \text{ and } r=.56, P<.05, \text{ respectively}) \), and HL \( (r=-.55 \text{ and } r=-.50, \text{ respectively, both } P<.05) \).

Percent LDL-II was not significantly correlated with any of the lipid and lipoprotein variables or associated enzymes (Table 3). However, the plasma concentration of LDL-II was positively associated with LDL-C concentrations, consistent with the fact that this was the major subfraction in the majority of individuals. This correlation was significant only in women \( (r=.79, P<.005) \) in whom the plasma concentrations of LDL-II and apoB were also associated \( (r=.73, P<.005) \).

Although percent LDL-III was not significantly correlated with any of the lipid and lipoprotein variables or associated enzymes, the concentration of this subfraction was positively associated with plasma cholesterol, LDL-C, and apoB concentrations and with HL activity (Table 3). These relations were present only in men \( (r=.5, r=.56, r=.59, \text{ and } r=.52, \text{ respectively, all } P<.05) \) when HL was also correlated with percent LDL-III \( (r=.49, P<.05) \). The percentage and concentration of LDL-III were significantly correlated with Lp(a) concentrations in women \( (r=.55 \text{ and } r=.54, \text{ respectively, both } P<.05) \) but not in men.
TABLE 3. Univariate Correlation Coefficients of Total Plasma LDL and Its Subfraction Distributions

<table>
<thead>
<tr>
<th>Subfraction</th>
<th>Chol</th>
<th>TG</th>
<th>VLDL-C</th>
<th>VLDL-TG</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>ApoA-I</th>
<th>ApoB</th>
<th>Lp(a)</th>
<th>LPL</th>
<th>HL</th>
<th>CETP</th>
<th>HDL2</th>
<th>HDL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total LDL, mg/dL</td>
<td>.91*</td>
<td>.48*</td>
<td>.37</td>
<td>.51*</td>
<td>.95t</td>
<td>-.20</td>
<td>-.03</td>
<td>.87t</td>
<td>-.08</td>
<td>-.01</td>
<td>.43t</td>
<td>.07</td>
<td>-.44t</td>
<td>-.10</td>
</tr>
<tr>
<td>LDL-I, %</td>
<td>-.33</td>
<td>-.49t</td>
<td>-.47t</td>
<td>-.65t</td>
<td>-.50t</td>
<td>.68t</td>
<td>.38</td>
<td>-.60t</td>
<td>.06</td>
<td>.24</td>
<td>-.60t</td>
<td>.06</td>
<td>.76t</td>
<td>.14</td>
</tr>
<tr>
<td>mg/dL</td>
<td>.04</td>
<td>-.37</td>
<td>-.42</td>
<td>-.54t</td>
<td>-.19</td>
<td>.63t</td>
<td>.36</td>
<td>-.34</td>
<td>.07</td>
<td>.24</td>
<td>-.50t</td>
<td>.05</td>
<td>.64t</td>
<td>.10</td>
</tr>
<tr>
<td>LDL-II, %</td>
<td>-.19</td>
<td>-.16</td>
<td>-.18</td>
<td>-.04</td>
<td>-.11</td>
<td>-.12</td>
<td>-.27</td>
<td>-.24</td>
<td>-.15</td>
<td>-.17</td>
<td>.11</td>
<td>-.10</td>
<td>-.22</td>
<td></td>
</tr>
<tr>
<td>mg/dL</td>
<td>.37</td>
<td>.08</td>
<td>.00</td>
<td>.21</td>
<td>.45*</td>
<td>-.14</td>
<td>-.19</td>
<td>.27</td>
<td>-.15</td>
<td>-.13</td>
<td>.08</td>
<td>.15</td>
<td>-.31</td>
<td>-.22</td>
</tr>
<tr>
<td>LDL-III, %</td>
<td>.23</td>
<td>.20</td>
<td>.15</td>
<td>.25</td>
<td>.28</td>
<td>-.19</td>
<td>-.01</td>
<td>.36</td>
<td>.27</td>
<td>.03</td>
<td>.41</td>
<td>.07</td>
<td>.25</td>
<td>.22</td>
</tr>
<tr>
<td>mg/dL</td>
<td>.48t</td>
<td>.32</td>
<td>.24</td>
<td>.35</td>
<td>.53t</td>
<td>-.22</td>
<td>-.01</td>
<td>.58t</td>
<td>.22</td>
<td>.02</td>
<td>.48t</td>
<td>.06</td>
<td>.35</td>
<td>.16</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; Chol, cholesterol; TG, triglyceride; VLDL-C, very-low-density lipoprotein cholesterol; LDL-C, LDL cholesterol; HDL-C, high-density lipoprotein cholesterol; Apo, apolipoprotein; Lp(a), lipoprotein(a); LPL, lipoprotein lipase; HL, hepatic lipase; CETP, cholesteryl ester transfer protein; and HDL, high-density lipoprotein. Pearson product-moment correlation coefficients are shown and were obtained using transformed data where appropriate. The significance of relations was tested by linear regression analysis.

*P<.01, tP<.005, tP<.0005.

Predictors of LDL Subfractions

The variability in LDL subtraction distribution for the whole study group was examined in statistical models to test for interactions between variables and identify independent predictors that might be important physiological regulators of subtraction distribution. In a first set of multivariate models we assessed the extent to which the distribution of LDL subfractions was influenced by body and lifestyle factors. As noted above, the men and women in this study differed principally with respect to LDL-I, and sex accounted for 28.8% of the variation in percent LDL-I (P<.001) and 26.6% of that in its concentration (P<.005). After correcting for the other factors (age, BMI, blood pressure, and smoking status), sex remained an independent predictor of percent LDL-I (r²=20.4%, P<.01) and its concentration (r²=17.3%, P<.05). Using a similar model that included sex, age, BMI, blood pressure, and smoking status as predictors, we found that the concentration but not percent of LDL-II was predicted by BMI (r²=13.0%, P<.05), despite the fact that BMI was not a univariate correlate of this subfraction. Neither the percentage nor concentration of LDL-III was predicted by any of the body and lifestyle factors.

In a second series of analyses, interactions between plasma lipid and lipoprotein variables were tested by including cholesterol, triglyceride, VLDL-C, VLDL-TG, LDL-C, HDL-C, Lp(a), apoA-I, and apoB in the model. None of these parameters were independent predictors of the percentage or concentration of LDL-I. The percentage and concentration of LDL-II were predicted by plasma VLDL-TG (r²=9.8% and r²=10.1%, respectively, both P<.05), LDL-C (r²=8.3%, P<.05 and r²=15.7%, P<.005, respectively), and apoB (r²=39.3% and r²=31.6%, respectively, both P<.0005) concentrations. The plasma apoB concentration was the only independent predictor of LDL-III percentage and concentration (r²=17.8% and r²=14.0%, respectively, both P<.05).

In a third series of models, the impacts of CETP, LPL, and HL were tested by including the three enzymes as predictors. Neither CETP nor LPL was a predictor of any of the LDL subtraction parameters, whereas HL was a strong independent predictor of the percentage and concentrations of LDL-I (r²=35.4%, P<.0005 and r²=23.9%, P<.005, respectively) and LDL-III (r²=16.9%, P<.05 and r²=23.3%, P<.01, respectively).

These observations were then extended by incorporating the independent predictors identified in the three series of multivariate analyses above together with other univariate correlates of the subfractions. The models were constructed so that they comprised only those predictors that continued to have a significant independent impact on the distribution of LDL subfractions.

When the two predictors of LDL-I, namely, sex and HL activity, were included in the same model (Table 4, LDL-I model a), the contribution of sex to the variation in percentage and concentration of LDL-I decreased from 29% and 27%, respectively, to 6% and 8%, respectively. The HL-independent contribution of sex was not statistically significant, indicating that the major impact of sex on the distribution of LDL-I was mediated through male-female differences in the activity of HL. The other univariate correlates of LDL-I (with the exception of HDL-C and HDL2 for the reasons outlined below) were then included in turn with HL in the multivariate model. Plasma triglycerides, VLDL-TG, and apoB (Table 4, LDL-I models b through d), but not VLDL-C or LDL-C, were HL-independent predictors of percent LDL-I. The model that explained most of the variation (approximately 37%) in percent LDL-I included HL and plasma triglycerides. Neither plasma triglycerides, VLDL-TG, nor apoB concentrations were significant HL-independent predictors of the concentration of LDL-I, although the model with HL and plasma triglycerides accounted for 27% of the variability in this subfraction parameter.

Although BMI, VLDL-TG, LDL-C, and apoB were identified as independent predictors of LDL-II in the series of analyses above, the contribution of BMI was not significant when the four variables were combined in the same model (Table 4, LDL-II model a). This suggested that the impact of BMI on LDL-II was mediated through the lipids, and when excluded from the model, more than 75% of the variation in the percentage and concentration of LDL-II was explained by the independent effects of LDL-C, apoB, and VLDL-TG (Table 4, LDL-II model b).
Plasma apoB concentrations and HL activity were identified as independent predictors of LDL-III and when included with Lp(a) in the same model explained more than 30% of the variation in the percentage and concentration of LDL-III (Table 4, LDL-III model a). However, apoB concentrations were not predictive of percent LDL-III, and when this parameter was excluded from the model, the predictive value of Lp(a) and HL increased such that they explained more than 43% of the variation in percent LDL-III and 47% of that in its concentration (Table 4, LDL-III model b).

### Coordinate Regulation of LDL and HDL Subfractions

The univariate analyses identified HDL-C and HDL2 as strong correlates of both the percentage and concentration of LDL-I (Fig 3). When HDL2 was included in the multivariate models of this subfraction, the impacts of sex and most significantly HL were lost, as this became the sole explanatory variable (Table 5, LDL-I model a). On univariate analysis, HDL2 explained 57% of the variation in percent LDL-I and 40.5% of that in its concentration (LDL-I model b). Conversely, in multivariate analyses of HDL2 levels, LDL-I accounted for 14% of its variability, while HL continued to make an independent contribution (Table 5). Whenever HL was included in the HDL2 analyses, sex had no independent effect, confirming that the male-female differences in LDL and HDL subfractions, as well as much of the relation between LDL-I and HDL2, were due to the activity of HL. Evidence for coordinate regulation of the small, dense LDL and HDL subfractions was also encountered, as LDL-III levels were predictive of HDL3 concentrations.

### Discussion

The purpose of the present study was to identify potential regulatory determinants of the percent distribution and plasma concentration of three discrete LDL subfractions. We chose young healthy subjects to examine how these subfractions are regulated in people with a preponderance of large LDL species (LDL-I and LDL-II), corresponding to the LDL pattern A previously described, as opposed to those individuals at risk of coronary artery disease who have an excess of small, dense LDL (LDL-III, pattern B). We were specifically interested in LDL-I, the levels of which are highest in young women and can be accurately measured by the method that we used, in contrast to previous studies that have used gradient gel electrophoresis or a variable calculated from the relative percentages of LDL bands identified on density gradient ultracentrifugation.

Three significant features emerged from the study: (1) HL is a key determinant of LDL subfraction distribution and is apparently responsible for sex differences in...
LDL-I, (2) neither LPL nor CETP plays a significant part in determining LDL heterogeneity in young normolipidemic individuals, and (3) there appears to be coregulation of LDL and HDL subfractions, suggesting that the atherogenic risk associated with each should not be considered independent of one another.

The women in this study had higher concentrations of LDL-I and HDL$_2$ than the men, and it has been suggested that this is due to the fact that the activity of HL in women is approximately two thirds of that found in men. We confirmed this in multivariate analyses in which the influence of sex ($r^2$) on LDL-I and HDL$_2$ concentrations was reduced from 27% to 8% and from 22% to 7%, respectively, when HL was included as a covariate. Although it is well established that plasma HDL$_2$ levels are regulated by HL, the impact of the enzyme on the metabolism of LDL subfractions has yet to be accepted. Evidence that HL might be important is shown in humans with HL deficiency who have a preponderance of light LDL species. Recently it has been suggested that HL might act across the LDL spectrum, as both the size and buoyancy of LDL were shown to be inversely related to HL activity in a group of men comprising coronary artery disease patients and normolipidemic subjects. Our results are consistent with this and indicate that HL may redistribute LDL-I to LDL-III, thus explaining the excesses of LDL-I in women and LDL-III in men and the inverse relation between LDL-I and LDL-III. Whether LDL-II is an intermediate in this conversion was not clear because HL was not a predictor of either its distribution or concentration. The conversion of LDL-I to LDL-III may be due to the lipolytic activity of HL, as suggested by the inverse relation between LDL triglyceride and HL.

Previous studies have shown that the peak LDL particle diameter is inversely related to plasma triglyceride concentrations, and we showed here that triglycerides, and VLDL-TG in particular, are important predictors of LDL-I and LDL-II. However, we found no relation between plasma triglycerids and small, dense LDL. This may have been due to the fact that our subjects covered a narrow range of plasma triglycerides (0.50 to 2.05 mmol/L; mean, 0.96 mmol/L) compared with the studies of healthy individuals reported by McNamara et al ($<4.5$ mmol/L), Swinkels et al ($<3.5$ mmol/L), and Williams et al ($<5.6$ mmol/L). It is possible that plasma triglycerides influence the distribution of denser LDL subfractions only once a certain threshold has been breached. In support of this, Austin et al found that individuals with triglycerides above 1.1 mmol/L were more likely to have the pattern B phenotype for LDL characterized by a predominance of small, dense LDL, while those below this threshold tended to have larger LDL. In addition, it has been found that the LDL subfraction pattern in individuals with raised triglycerides is changed from pattern B to pattern A when their plasma triglycerides are reduced to less than 1.5 mmol/L.

It has been suggested that high plasma triglycerides influence LDL through a cycle of lipid exchange, so that LDLs become enriched in triglyceride and are then acted on by the lipases to produce a population of small, dense, lipid-poor LDL. Although CETP appears capable of modifying LDL composition and size in vitro, neither Karpe et al nor our group found that its plasma activity correlated with LDL subfraction distri-

---

**Table 5. Coordinate Regulation of LDL and HDL Subfractions**

<table>
<thead>
<tr>
<th>Subfraction Model</th>
<th>Predictor*</th>
<th>Percentage</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-I</td>
<td>a</td>
<td>Sex</td>
<td>$r^2$, %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HL (-)</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HDL$_2$ (+)</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>HDL$_2$ (-)</td>
<td>57.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LDL-I, mg/dL (+)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HL (-)</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cholesterol (-)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ApoA-I (+)</td>
<td>40.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LDL-III, mg/dL (+)</td>
<td>8.7</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; HL, hepatic lipase; and Apo, apolipoprotein. Multivariate analyses of percentage and plasma concentrations of LDL and HDL subfractions were obtained using general linear models. The independent contribution of the variable to the variation in each subfraction parameter ($r^2$) was calculated.

*Direction of predictive value.
Coordinating regulation of the levels of LDL-I and HDL-2.

Individuals. Specifically, we have shown that HL and plasma apoB, suggesting that distribution of this fraction might be regulated chiefly by factors that control these variables, namely, the synthesis of apoB and LDL and the clearance of LDL from the circulation. ApoB was also a predictor of LDL-III concentrations, and this may be attributable to apoB synthesis rather than LDL catabolism because pharmacologic stimulation of LDL receptor activity with cholesterylamine or simvastatin has been shown to have no effect on LDL-III levels. The relation between Lp(a) and LDL-III could possibly be explained if this LDL subtraction was contaminated with Lp(a). In contrast, we have found only trace amounts of Lp(a) (<2 mg/dL) in LDL-III isolated from subjects with high Lp(a) concentrations (>80 mg/dL) (M.J.C., C.J.P., J.S., 1994, unpublished data), so that it is probable that either (1) LDL-III and Lp(a) are controlled by similar mechanisms or (2) Lp(a) is acting as a marker of another, unmeasured, factor that influences LDL-III.

Coordinate changes in LDL and HDL subfractions have been reported previously in healthy men when positive correlations were found between buoyant LDL and HDL(1) and between dense LDL and HDL(2). These trends were substantiated in the present study of men and women, which identified HDL(2) as a strong predictor of LDL-I, and vice versa, and LDL-III as a weaker determinant of HDL(2). The metabolism of LDL-I and HDL(2) appears to be linked through the activity of HL, and the close relation between these two subfractions raises the possibility that the cardioprotective effect of HDL(2) might be in part due to its association with a less atherogenic LDL phenotype. In conclusion, we have identified a number of potential regulators of LDL- and HDL-2 subclasses in healthy men.

Acknowledgments

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