Low Density Lipoprotein Subfractions and Relationship to Other Risk Factors for Coronary Artery Disease in Healthy Individuals

Dorine W. Swinkels, Pierre N.M. Demacker, Jan C.M. Hendriks, and Albert van 't Laar

By a recently developed sensitive density gradient ultracentrifugation method, the distribution of low density lipoprotein (LDL) subfractions was studied in the serum of healthy blood donors (20 to 62 years old). For each subject, we observed a specific LDL subtraction distribution characterized by the relative contribution of the three major LDL subfractions, LDL-1 (1.020 to 1.028 g/ml), LDL-2 (1.027 to 1.034 g/ml), and LDL-3 (1.033 to 1.039 g/ml), to total LDL. Statistical analysis was performed by using the LDL density variable defined as: (% of LDL-1) x 1.024 + (% of LDL-2) x 1.0305 + (% of LDL-3) x 1.036 as a continuous variable. Controlling for age, smoking habits, relative body weight and, when appropriate, for gender, it appeared that: 1) dense LDL subtraction patterns characterized by a predominant LDL-3 subtraction and a decreased LDL particle size were more likely to be found among men than among women, 2) with increasing density of LDL, the levels of serum triglycerides increased, whereas the concentration of HDL cholesterol and the ratio of LDL cholesterol to LDL apolipoprotein (apo) B decreased, and 3) the best model with significant contribution in the prediction of the LDL subtraction distribution was the three-variable model: total cholesterol, serum triglycerides, and LDL apo B ($R^2$=0.40), whereas the best two-variable model consisted of serum triglycerides and high density lipoprotein cholesterol ($R^2$=0.37). These data are consistent with results from a study described previously in which a different approach based on LDL subtraction quantification by gradient gel electrophoresis of whole plasma was used. (Arteriosclerosis 9:604–613, September/October 1989)

Plasma levels of low density lipoproteins (LDL) are positively correlated with the prevalence of coronary artery disease.1 By various methods, LDL have been found to be heterogeneous, consisting of subfractions that differ in physicochemical characteristics.2-7 This raises the question of whether the various LDL subfractions differ in their ability to induce atherosclerosis. Evidence that the distribution of LDL subfractions has a genetic base8,9 has been found, and two phenotypes, characterized by a major peak of LDL subclass with either large or small diameters, can be distinguished with gradient gel electrophoresis.9 The subjects with either phenotype, however, had serum lipid and lipoprotein concentrations that were significantly different, raising the question of whether the LDL subclass pattern is primary or secondary to these differences. It has been suggested that one of the phenotypes may represent familial combined hyperlipoproteinemia or hyperapobetalipoproteinemia, both of which are characterized by an increased risk for coronary artery disease.10-11

To study the relationship between LDL subtraction distribution and serum lipids, apolipoproteins, and LDL apolipoprotein (apo) B, LDL subfractions were analyzed and quantitated in the sera of 62 male and 69 female blood donors by a recently developed, sensitive, density gradient ultracentrifugation method.7 Different LDL subtraction patterns emerged, and serum lipids and lipoproteins were determined and compared to gain insight into the possible metabolic factors that underly the heterogeneity of LDL. In the discussion, we compare our results with those obtained recently by McNamara et al.,12 who separated LDL subfractions by gradient gel electrophoresis of whole plasma.

**Methods**

**Study Protocol**

Blood from 146 apparently healthy blood donors ages 20 to 62 years was sampled. The subjects had not fasted, but blood was drawn within 2 hours after a light continental breakfast. Under these conditions, chylomicrons are expected to be absent in normolipidemic persons; the variation in serum triglycerides due to the nonfasting...
condition does not exceed the biological variation of 25% in sera after 10 hours of fasting. Sera were isolated within 2 hours. The subjects had filled out questionnaires identifying the most important risk factors for coronary heart disease and other factors that influence serum lipids and lipoproteins (drugs used, smoking habits, height and weight, age, and physical activity). Relative body weight was calculated according to the Metropolitan Life Insurance Company 1983 tables. The physical activity of the subjects was low or moderate. Subjects using β-blockers or diuretics (n=5), and subjects with total serum cholesterol >7.75 or triglyceride >3.50 mmol/l (n=7), were excluded from the study. In addition, one obese woman, whose relative body weight was 194%, was also excluded. The resulting study population consisted of 62 men and 69 women.

Detection of Low Density Lipoprotein Subfractions

The LDL banding pattern was analyzed as described: 3.4 ml of serum was prestained with Coomassie brilliant blue R (CBBR) and was ultracentrifuged in a density gradient at 160 000 g at 20°C for 19.5 hours in an IEC SW 41 rotor (no. 488, 6×14 ml) in an IEC-B60 ultracentrifuge (Damon/IEC, Needham Heights, MA) or in an MSE Pre-spin 75 ultracentrifuge with an MSE Ti 40 rotor (catalogue no. 43127-111). After ultracentrifugation, an almost linear density gradient was obtained with up to three major LDL subfractions in the density range of 1.020 to 1.039 g/ml (between fractionation marks 8.5 and 12), very light LDL-1 (1.020 to 1.028 g/ml), light LDL-2 (1.027 to 1.034 g/ml), and heavy LDL-3 (1.033 to 1.039 g/ml).

Extending the ultracentrifugation time from 19.5 to 39 hours resulted in LDL bands that: 1) were located further apart, that is, LDL-1 moved slightly toward the top of the tube, whereas LDL-2 and LDL-3 moved slightly toward the bottom; and 2) were less sharp, that is, the LDL peaks became broader and the peak heights decreased.

The effect of the prestaining procedure was previously evaluated by means of density gradient ultracentrifugation of pooled sera. The profile of the cholesterol content along the gradient was determined in the tube after ultracentrifugation of both nonstained serum and serum prestained with CBBR. It appeared that the staining procedure did not result in a change of LDL density. In addition, density gradient ultracentrifugation was performed in the same run with two nonstained and two stained tubes. When three subfractions were observed, the nonstained subfractions were yellow, whereas the stained subfractions colored blue. It appeared that for nonstained samples, the LDL bands were, in fact, visible, and the number of bands, their position, and their chemical composition were similar in both methods. However, because of the yellow in the nonstained subfractions, some experience in visually differentiating these subfractions is needed. Furthermore, the staining procedure allows better recording of the LDL banding pattern by photography and densitometry.

In 50 out of 131 sera, one (n=42), two (n=7), or three (n=1) additional minor bands (less than 5% of the densitometric absorbance of the total LDL band) in the density range of 1.039 to 1.061 g/ml (between the fractionation marks 12 and 15.5) were detected. The sera of six of the 50 individuals with polydisperse sera were ultracentrifuged again in the density gradient, and a total of twelve minor bands emerged (one serum showed one minor LDL subfraction, four sera showed two minor LDL bands, and one serum showed three minor bands). These minor bands were then isolated and characterized by polyacrylamide gradient gel electrophoresis and by immunodiffusion on 0.8% agarose by using specific antisera (from sheep) against the Lp(a) protein.

After ultracentrifugation, the tubes were placed in a specially designed rack and were photographed. The following conditions were used to obtain optimal photographs: lens, 135 mm (Olympus, OM-1N); aperture, f/11; shutter speed, 1/30 seconds; filter, Kodak Wratten nos. 81 and 30 m; illumination, 15 cm behind the tubes from a diffuse light source (illumination for X-rays); distance, 45 cm; film, Kodak Ektachrome 200 Professional Daylight 135-36, process-E6.

LDL subfractions were quantitated by densitometric scanning of the slides on an LKB 2202 ultrascan laser densitometer, beamsize 0.8×0.05 mm with a gaussian distribution. The scan was started at fractionation mark 12.5 and was ended at fractionation mark 6. Peak identification, integration of peak areas, and calculation of the relative contribution of each peak area to the total LDL band was performed by the LKB 2190 GelScan program on an Apple Ile computer. For peak identification, the peak width was set so that the number of peaks found by an automatic search of the GelScan program agreed with those seen in the tube. The program then started to fit each peak by using a gaussian curve (inaccuracy ≤5%). The area under each curve was calculated and expressed as a percentage of the total LDL band. Repeating this computerized modeling procedure for the same densitometric scan resulted in identical results.

Analysis of Lipoproteins and Apolipoprotein B

The density of 5 ml of serum was raised to d=1.019 g/ml by the addition of D2O (d=1.10 g/ml). Very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) were isolated by ultracentrifugation for 16 hours at 40 000 rpm (168 000 g) in an IEC B-60, fixed-angle rotor no. 488 (Damon/IEC). High density lipoprotein (HDL) cholesterol was determined in whole serum by the polyethylene-glycol 6000 method. LDL cholesterol was calculated by subtracting VLDL+IDL cholesterol and HDL cholesterol from total serum cholesterol. Apo B in the d>1.019 g/ml fraction was determined in duplicate plates by radial immunodiffusion in 0.8% (wt/vol) agarose in a barbital buffer (pH=8.6). The 0.8% (wt/vol) agarose contained 0.3% (vol/vol) anti-apo B antisera, which was raised in rabbits against LDL (1.030 to 1.050 g/ml). When the duplicates differed by more than 10%, radial immunodiffusion was repeated.

Statistical Analysis

Statistical analysis was performed with LDL density defined as: (% of LDL-1)x1.024+ (% of LDL-2)x1.0305+ (% of LDL-3)x1.036 as a continuous variable. In this
equation, the weighted factors, 1.024, 1.0305, and 1.036, represent the midpoints of the density intervals of the LDL subfractions, LDL-1, LDL-2, and LDL-3, respectively.

Multiple linear regression was performed to determine the influence of gender, age, relative body weight, and smoking habits (independent variables) on LDL density (dependent variable).

A different multiple linear regression was performed to examine the influence of LDL density, gender, age, relative body weight, and smoking habits (independent variables) on serum cholesterol, serum triglycerides, HDL cholesterol, LDL apo B, LDL cholesterol, and the ratio of LDL cholesterol/LDL apo B (dependent variables).

Pearson correlation coefficients were computed to:  
1) determine correlations between the variables: serum cholesterol, serum triglycerides, HDL cholesterol, LDL cholesterol, LDL apo B, and LDL cholesterol/LDL apo B;  
2) identify among these same variables those having a significant correlation with the LDL density variable. The differences in the correlations obtained in the present study and those from a previous report by McMama et al.12 were tested for significance using Fisher's Z transformation.

Multiple linear regression model-selection techniques included forward selection, backward elimination, and a stepwise procedure; these were used to examine the

![Image](http://atvb.ahajournals.org/)

**Figure 1.** Low density lipoprotein (LDL) subfraction distribution in healthy subjects. A. LDL banding patterns after density gradient ultracentrifugation for six individuals. Marks on the tube facilitate the layering: 1, 2, and 3 represent LDL-1, LDL-2, and LDL-3, respectively. Fractionation marks on tube A are used to determine the density of the LDL subfractions. Note that the photography causes a loss of sensitivity. B. LDL subfraction distribution determined by densitometric scanning of the tubes shown in A, followed by computerized mathematical modeling. The solid-line curves were obtained by densitometric scanning of the bands visible on the slides taken of the tubes. The interrupted-line gaussian curve was determined by deconvolution analysis of the measured curve. The dotted-line curve represents the sum of the composing gaussian curves. The peak densities of the gaussian curves are indicated. From subject A to subject F (left to right), the contributions to total LDL of LDL-1 are 8.2%, 7.8%, 20.3%, 18.3%, 42.6%, and 64.0%; of LDL-2, the contributions are 13.0%, 62.0%, 34.8%, 63.2%, 27.6%, and 15.0%; and of LDL-3, the contributions are 78.8%, 30.3%, 44.9%, 18.4%, 29.9%, and 21.0%, respectively. From subject A to subject F, the values of the LDL density variable [defined as (% of LDL-1) x 1.024 + (% of LDL-2) x 1.0305 + (% of LDL-3) x 1.036] are 103.430, 103.269, 103.165, 102.929, 103.041, and 102.749, respectively.
significant contributions of the independent variables (total cholesterol, total triglycerides, HDL cholesterol, LDL apo B, LDL cholesterol, and the ratio of LDL cholesterol/LDL apo B) to the prediction of the LDL density variable. Any influence of gender, age, smoking habits, and relative body weight was taken into account by including them in each model.


Reproducibility of Quantification of Low Density Lipoprotein Variable

The sera of 10 blood donors were ultracentrifuged in duplicate at the same time in two different tubes placed in two different swinging bucket rotors. Both tubes were then photographed separately, and the LDL subfractions were quantitated by densitometric scanning as described above. The mean values for the LDL density variable (±SD) were 103.08±0.16 and 103.12±0.15. The mean difference (±SD) in the value of the LDL density variable between the duplicates was 0.04±0.09. There was no systematic error in the calculated LDL density variable between the two rotors. A one-way analysis of variance was performed with the LDL density variable as the dependent and subject as the independent variable to estimate the between- and within-subject variations. The SD between subjects (within population) and within subjects (measurement error) were estimated as 0.141 and 0.067, respectively. The between subjects/within subjects SD ratio of approximately 2 (reflecting a correlation coefficient of 0.83 between the duplicates of the LDL density variables) is acceptable for detecting differences within the studied population.

Results

The number of major LDL subfractions found for the 131 individuals was two (n=16) or three (n=115) in the density range of 1.020 to 1.039 g/ml: LDL-1 (1.020 to 1.028 g/ml), LDL-2 (1.027 to 1.034 g/ml), and LDL-3 (1.033 to 1.039 g/ml) (Figure 1). As described in the Methods section, in 50 sera, one (n=42), two (n=7), or three (n=1) additional minor bands in the density range of 1.039 to 1.061 g/ml were detected. For six sera, a total of 12 minor bands were isolated, which were characterized as LDL-4 (n=2), Lp(a) (n=4), or LDL-4 (n=1) and LDL-5 (n=5), both "contaminated" with Lp(a). The minor LDL bands, LDL-4 and LDL-5, were arbitrarily defined on the basis of increasing density (1.039 to 1.049 g/ml vs. 1.049 to 1.061 g/ml)
Figure 3. A triangular presentation of the contribution of the three major low density lipoprotein (LDL) subfractions, LDL-1, LDL-2, and LDL-3, to the total LDL in the 131 healthy subjects. Each square represents the LDL subfraction distribution of one individual. The length of the sides of the squares is directly proportional to the value of LDL density defined as: (% of LDL-1) x 1.024 + (% of LDL-2) x 1.0305 + (% of LDL-3) x 1.036.

to 1.061 g/ml) and decreasing size. Figure 2 illustrates the presence of minor subfractions in the density gradient and in the gradient gel electrophoresis for one donor; Lp(a) was present in two of the three minor subfractions, and, at increasing density, the minor LDL bands were of decreasing size.

For each individual, the LDL subfraction distribution was determined by the relative contribution of the major subfractions, LDL-1, LDL-2, and LDL-3 to total LDL (Figure 3). This LDL subfraction distribution was approached by the LDL density variable constructed from the weighted average of the relative percentages of the three observed major LDL bands: (% of LDL-1) x 1.024 + (% of LDL-2) x 1.0305 + (% of LDL-3) x 1.036. In Figure 3, each individual is represented by a square, and the length of the sides of each square is directly proportional to the calculated value for the LDL density variable. A low value for the LDL density variable (small squares) was obtained for subjects with a predominant LDL-1 fraction, whereas a high value (large squares) was obtained for individuals with a predominant LDL-3 fraction. Furthermore, an enhanced LDL density variable indicated a preponderance of relatively small LDL particles (Figure 2). The frequency of the LDL density variable among the 131 subjects showed a normal distribution (Figure 4).

The characteristics and the lipid and apolipoprotein levels for the subjects are shown separately for men and women in Table 1. Minor LDL bands were seen more often in men than in women. The serum triglyceride and HDL cholesterol variables correlated significantly (p<0.05) with the LDL density variable in both men and women. Moreover, increased LDL apo B levels correlated significantly with denser LDL for men. Although nonsignificant, note the correlation (0.05<p<0.10) of a more dense LDL subfraction pattern with increased smoking habits in women and a decreased ratio of LDL cholesterol to LDL apo B in men.

Multiple linear regression revealed that gender had a significant (p<0.05) influence on the LDL density variable (Table 2). This indicates that men are more likely than women to have heavy LDL.

To examine the influence of the LDL density variable on lipids, lipoproteins, and LDL apo B, a multiple linear regression model, including the interaction between LDL density and age, smoking habits, relative body weight, and gender, was carried out. No significant interactions...
were found, indicating that the influence of LDL density on lipids, lipoproteins, and LDL apo B did not depend on age, number of cigarettes smoked, relative body weight, or gender. For this reason, only the model with main effects is presented. This model showed a significant influence of LDL density on the concentrations of serum triglycerides ($p<0.001$) and HDL cholesterol ($p<0.001$) and on the ratio of LDL cholesterol to LDL apo B ($p<0.05$), independent of the influences of other risk factors such as age, smoking habits, relative body weight, and gender (Table 3). Hence, dense LDL was accompanied by enhanced triglyceride and HDL cholesterol levels and a decreased ratio of LDL cholesterol to LDL apo B.

The stepwise multiple regression analysis included gender, age, relative body weight, and smoking habits; only variables that, in addition to these risk factors, contributed significantly to the prediction of LDL density were evaluated. Total cholesterol, serum triglycerides, and LDL apo B appeared to make a significant contribution to the prediction of LDL density. No other variable reached the $p=0.05$ level of significance. This three-variable model is the best model for prediction of LDL density ($R^2=0.40$). The best two-variable model ($R^2=0.37$) for prediction of LDL density included serum triglycerides and HDL cholesterol. Table 4 gives the regression coefficients and their significance levels for the variables selected. Apparently, the combination of the variables LDL apo B and total cholesterol in the three-variable model was a slightly better predictor for variation in LDL density than was HDL cholesterol in the two-variable model.

These two- and three-variable models appeared to be the most appropriate for predicting LDL density. However, a series of other two- and three-variable models revealed one more two-variable model and four more three-variable models in which each variable contributed significantly to the prediction of LDL density (Table 5). For a better understanding of the relevance of the different models in the prediction of LDL density, we determined the correlations between variables (Table 6). The higher the correlation between two variables, the better the comparability between models in the prediction of LDL density when these variables replace each other. Thus, when total cholesterol and LDL apo B were independently replaced by

![Frequency distribution of low density lipoprotein (LDL) density in 131 healthy subjects indicating the values of the midpoints of the LDL density intervals. The median 103.038.](http://atvb.ahajournals.org/)

Table 1. Characteristics of Subjects and Correlations with Low Density Lipoprotein Density in this Study and in a Study by McNamara et al.12

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men</th>
<th>All</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>This study</td>
<td>Ref. 12 p</td>
<td>This study</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>62 (31)</td>
<td>131 (50)</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>39.0±11.3</td>
<td>0.11</td>
<td>0.17†</td>
</tr>
<tr>
<td>Body weight (%)</td>
<td>111±12</td>
<td>0.03</td>
<td>113±14</td>
</tr>
<tr>
<td>Smoking (cig/day)</td>
<td>6±10</td>
<td>0.13</td>
<td>5±9</td>
</tr>
<tr>
<td>LDL density</td>
<td>103.016±0.140</td>
<td>103.102±0.183</td>
<td>103.057±0.167</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.4±1.0</td>
<td>0.08</td>
<td>0.28‡</td>
</tr>
<tr>
<td>Total triglyceride</td>
<td>1.6±0.7</td>
<td>0.47§</td>
<td>0.79§</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.2±0.3</td>
<td>-0.506</td>
<td>-0.58§</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.4±0.8</td>
<td>0.15</td>
<td>0.70</td>
</tr>
<tr>
<td>LDL apo B (g/l)</td>
<td>1.0+0.37</td>
<td>0.25†</td>
<td>0.98±0.32</td>
</tr>
<tr>
<td>LDL-C/LDL apo B (mmol/g)</td>
<td>3.6±0.8</td>
<td>-0.13</td>
<td></td>
</tr>
</tbody>
</table>

Values are given in mmol/l unless otherwise indicated. Pearson correlations and their levels of significance of the indicated variables with the LDL density variable for this study and a previous report12 are given. The $p$ value is the difference in correlation between studies.

*The numbers in parentheses are the number of subjects with at least one minor band in the density range of 1.039 to 1.061 g/ml. 10.01< p<0.05, 10.001< p<0.01, 5p<0.001, ||p<0.010. Abbreviations: SD = standard deviation, cig = cigarettes, LDL = low density lipoprotein, HDL = high density lipoprotein, apo = apolipoprotein, LDL-C = low density lipoprotein cholesterol.
LDL cholesterol, this resulted in comparable, but less predictive, three-variable models (R²=0.35 and 0.38, respectively, Table 5).

**Discussion**

When single-spin density gradient ultracentrifugation and pre-staining of the serum was used, LDL appeared heterogeneous and consisted of various subfractions. Each subject had his/her own LDL subtraction pattern, which was characterized by the distribution of LDL among three major LDL subfractions in the density range of 1.020 to 1.039 g/ml. Similar results were obtained with gradient gel electrophoresis of total serum and 2% to 16% polyacrylamide gels (Swinkels DW and Demacker PNM, unpublished data).

The major LDL subfractions, LDL-1 (1.020 to 1.028 g/ml), LDL-2 (1.027 to 1.034 g/ml), and LDL-3 (1.033 to 1.039 g/ml) detected in the present study are actually more buoyant and appear to be concentrated in a narrower density range than are the LDL subtraction groups I (1.025 to 1.035 g/ml), II (1.035 to 1.040 g/ml), III (1.040 to 1.050 g/ml), and IV (1.050 to 1.060 g/ml) found by Krauss and Burke after equilibrium density gradient ultracentrifugation of LDL from normolipemic subjects. However, the density boundaries of the most frequently occurring LDL subtraction reported here (LDL-2, d=1.027 to 1.034 g/ml) and recently by Chapman et al. in a similar gradient at 15°C (LDL-7, d=1.0297 to 1.0327) appear comparable.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>LDL density variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (x10⁻³)</td>
<td>-0.727±0.307†</td>
</tr>
<tr>
<td>Age (x10⁻²)</td>
<td>0.937±1.465</td>
</tr>
<tr>
<td>Relative body weight (x10⁻⁵)</td>
<td>-0.690±1.143</td>
</tr>
<tr>
<td>Smoking (x10⁻²)</td>
<td>0.289±0.162</td>
</tr>
<tr>
<td>Intercept</td>
<td>103.197±0.128</td>
</tr>
</tbody>
</table>

*Men=1, women=2.

The significance of the influence of independent variable on dependent variable: *0.05<p<0.10, †0.01<p<0.05, ‡0.001<p<0.01, §p<0.001.

HDL = high density lipoprotein, LDL = low density lipoprotein, apo = apolipoprotein.

In our study, the bands with a density higher than 1.039 g/ml (1.039 to 1.061 g/ml) contributed less than 5% to the total LDL band and were, therefore, depicted as minor fractions and were excluded from the analyses. These observations correspond with those of Krauss and Burke who used gradient gel electrophoresis of the d<1.063 g/ml lipoprotein fraction. These authors noted that the small LDL particles (size region F, 23.2 to 24.3 nm) are observed frequently but always as minor species in normal subjects. In a recent report by McNamara et al., however, 29% of the normal subjects studied appeared to have LDL subfractions with a density >1.038 g/ml (LDL-4 to LDL-7) as their most intense LDL subfraction band after gradient gel electrophoresis of total plasma. These apparent discrepancies can only be elucidated by comparing the quantitative LDL subfraction distribution measured by both density gradient ultracentrifugation and gradient gel electrophoresis for a large number of normal subjects in one study.

The fractions with a density >1.039 g/ml usually reacted positively with antiseraum against Lp(a). Similar results were obtained for fractions 5 and 6 (d>1.050 g/ml) in a report by Krauss and Burke; for LDL layer 4 (d 1.046 to 1.054 g/ml) in the study of Lee and Downs, and for LDL fractions 11 to 15 (d>1.045 g/ml) in a recent work by Chapman et al.

The LDL subfractions were objectified by densitometric scanning of the prestained LDL bands. The curves thus obtained appeared similar to the densitometric gradient gel electrophoresis curves reported previously by Austin and Krauss and could also be deconvoluted into three components. This conformity in the detection of LDL subfractions by size (by gradient gel electrophoresis) and density (density gradient ultracentrifugation) supports the existence of LDL heterogeneity as a physiological phenomenon.

The peak areas were integrated and expressed as the percentage of the total LDL band. The LDL subfraction data thus obtained for the 131 subjects were shown to be of great interindividual variety, with three peaks in most subjects. Austin and Krauss, however, distinguished no more than two different LDL subfraction patterns, in which the major LDL peak represented either the smaller (<25.5 nm) or the larger (>25.5 nm) LDL particles. Although this subdivision in two LDL subclass patterns may be appro-
Table 4. Regression Coefficients and Levels of Significance for Variables in Models Most Appropriate for Predicting Low Density Lipoprotein Density

<table>
<thead>
<tr>
<th>Variable</th>
<th>Three-variable model</th>
<th>Two-variable model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression coefficient</td>
<td>p</td>
<td>Regression coefficient</td>
</tr>
<tr>
<td>Gender (×10^-1)</td>
<td>-0.226±0.273</td>
<td>0.41</td>
</tr>
<tr>
<td>Age (×10^-2)</td>
<td>0.063±0.1292</td>
<td>0.63</td>
</tr>
<tr>
<td>Relative body weight (×10^-3)</td>
<td>-1.760±0.985</td>
<td>0.08</td>
</tr>
<tr>
<td>Smoking (×10^-3)</td>
<td>0.247±0.136</td>
<td>0.07</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>-0.091±0.021</td>
<td>0.00</td>
</tr>
<tr>
<td>Total triglyceride (mmol/l)</td>
<td>0.154±0.023</td>
<td>0.00</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL apo B (mg/l ×10^-3)</td>
<td>0.168±0.057</td>
<td>0.00</td>
</tr>
<tr>
<td>Intercept</td>
<td>103.35</td>
<td></td>
</tr>
</tbody>
</table>

The R^2 for the three-variable model is 0.40; for the two-variable model, it is 0.37.

HDL = high density lipoprotein, LDL = low density lipoprotein, apo = apolipoprotein.

Table 5. Two- and Three-variable Regression Models that Predict Low Density Lipoprotein Density

<table>
<thead>
<tr>
<th>Model</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-variable model</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol and total triglyceride</td>
<td>0.35</td>
</tr>
<tr>
<td>HDL cholesterol and total triglyceride*</td>
<td>0.37</td>
</tr>
<tr>
<td>Three-variable model</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, HDL cholesterol, and LDL cholesterol</td>
<td>0.31</td>
</tr>
<tr>
<td>HDL cholesterol, LDL cholesterol, and LDL apo B</td>
<td>0.31</td>
</tr>
<tr>
<td>Total triglyceride, LDL cholesterol, and LDL apo B</td>
<td>0.35</td>
</tr>
<tr>
<td>Total cholesterol, total triglyceride, and LDL cholesterol</td>
<td>0.38</td>
</tr>
<tr>
<td>Total cholesterol, total triglyceride, and LDL apo B*</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Models in which variables, in addition to gender, age, smoking habits, and relative body weight, contribute significantly (p<0.05) to the prediction of LDL density.

*The best models for predicting LDL density.

HDL = high density lipoprotein, LDL = low density lipoprotein, apo = apolipoprotein.

The remainder may be due to factors not measured, that is, the activity of lipoprotein lipase, hepatic lipase, exchange proteins, lecithin-cholesterol acyltransferase activity, and genetic variation in apoprotein B.26-31
Men are more likely to have a dense LDL subtraction pattern than women (Table 2). In view of the enhanced serum triglyceride concentration and decreased HDL levels for men and the interrelations in the metabolism of the different lipoproteins, this is not surprising.

We found an association of an enhanced LDL density variable with a decreased ratio of LDL cholesterol to LDL apo B, indicating that, as the contribution of dense LDL (LDL-3) to total LDL increases, LDL become relatively more protein enriched. In the disorder of hyperapobetalipoproteinemia, which is strongly associated with atherosclerosis, such a protein-enriched LDL particle has also been described. This suggests that patients with hyperapobetalipoproteinemia may have increased LDL density.

Thus, variations in the LDL subtraction distribution are not independent of differences in other variables that are related to coronary artery disease, such as total cholesterol, total triglyceride, HDL cholesterol, LDL apo B, LDL cholesterol/LDL apo B ratio, smoking habits, and gender.

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