Associations of Lipoproteins and Apolipoproteins With Gradient Gel Electrophoresis Estimates of High Density Lipoprotein Subfractions in Men and Women

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We examined the relations of gender and lipoproteins to subclasses of high density lipoproteins (HDLs) in a cross-sectional sample of moderately overweight men (n=116) and women (n=78). The absorbance of protein-stained polyacrylamide gradient gels was used as an index of mass concentrations of HDL at intervals of 0.01 nm across the entire HDL particle size range (7.2-12 nm). At least five HDL subclasses have been identified by their particle sizes: HDL_1 (7.2-7.8 nm), HDL_2 (7.8-8.2 nm), HDL_3 (8.2-8.8 nm), HDL_4 (8.8-9.7 nm), and HDL_5 (9.7-12 nm). Men had significantly higher HDL_3 and significantly lower HDL_1 and HDL_5 than did women. Correlations of HDL subclasses with concentrations of other lipoprotein variables were generally as strong for gradient gel electrophoresis as for analytical ultracentrifugation measurements of HDL particle distributions. In both sexes, high levels of HDL_3 were associated with coronary heart disease risk factors, including high concentrations of triglycerides, apolipoprotein B, small low density lipoproteins, intermediate density lipoproteins, and very low density lipoproteins and low concentrations of HDL_2 cholesterol and HDL_2 mass. Plasma concentrations of HDL_4 cholesterol were unrelated to protein-stained HDL_3 levels. HDL_4 cholesterol concentrations also did not exhibit the sex difference or the relations with lipoprotein concentrations that characterized HDL_3. Thus, low HDL_2 levels may contribute in part to the low heart disease risk in men and women who have high HDL_4 cholesterol. Measurements of HDL_4 cholesterol may not identify clinically important relations involving HDL_3.

KEY WORDS • gradient gel electrophoresis • very low density lipoproteins • low density lipoproteins • high density lipoproteins • sex differences

High density lipoproteins (HDLs) include multiple distinct subclasses of particles that have different electrophoretic mobilities on non-denaturing polyacrylamide gradient gels. The different mobilities reflect the impeded migration through the gel of the larger HDL particles. Previous studies have described HDL subclasses by 1) the apparent size of the particles at the distribution’s major and minor peaks; and 2) the proportion of HDL protein occurring within intervals believed to contain predominantly HDL subclasses. In this report, we examine the relations of gender, plasma lipoproteins, and apolipoproteins to protein-stained HDL subclasses in a sample of moderately overweight subjects (116 men and 78 women). The absorbance of protein-stained gels is used as an index of mass concentrations of HDL at intervals of 0.01 nm across the entire HDL particle size range (7.2-12 nm). By examining each diameter value, we are able to identify the regions according to size that are associated with gender and variations in lipid and lipoprotein concentrations without necessarily assuming the shapes or particle size intervals of the HDL subclasses. We also assess whether HDL heterogeneity is adequately represented by HDL_2 and HDL_3 cholesterol measurements. In an “Appendix,” we assess the correspondence between estimated HDL particle diameter (gradient gel electrophoresis) and HDL flotation rate (analytical ultracentrifugation), and we compare the lipoprotein correlations obtained for absorbance of HDL protein (gradient gel electrophoresis) and in the gradient gel electrophoresis.
phoresis) with those obtained for plasma concentrations of HDL mass (analytical ultracentrifugation).

**Methods**

**Subjects**

We studied healthy nonsmoking men and women, aged 25 through 49 years. All were relatively sedentary (exercising no more than twice weekly), moderately overweight (body mass index between 28 and 34 kg/m² in men and between 24 and 30 kg/m² in women), free of medication that might affect lipid metabolism, nonhypertensive (blood pressure <160/95 mm Hg), and nonhyperlipidemic (plasma total cholesterol <260 mg/dl and triglycerides <500 mg/dl). The women were all premenopausal with normal menstrual cycles. None of the women were pregnant, lactating, or using oral contraceptives.

**Laboratory Methods**

All participants reported to our clinic in the morning, having abstained for 12–16 hours from all food and any vigorous activity. Venous blood was collected through a butterfly catheter into a syringe, with the subject in a supine position for less than 10 minutes. The blood was immediately transferred into tubes containing sodium EDTA (22.5 mg/15-ml tube). Plasma total cholesterol and triglyceride concentrations were measured by enzymatic methods (Abbott ABA 200 instrument, Abbott Diagnostics, North Chicago, Ill.), and HDL cholesterol was measured by dextran sulfate–magnesium precipitation, followed by enzymatic determination of cholesterol. These measurements were consistently in control as monitored by the Lipid Standardization Program of the Centers for Disease Control, Atlanta, Ga., and the National Heart, Lung, and Blood Institute, Bethesda, Md. HDL, cholesterol was determined by a dextran sulfate–magnesium precipitation method, and HDL, cholesterol was calculated as the difference between total HDL cholesterol and HDL, cholesterol. Plasma apolipoprotein (apo) A-I and B concentrations were determined by rate immunonephelometry (Beckman ARRAY, Beckman Instruments, Palo Alto, Calif.). Subjects were classified into low density lipoprotein (LDL) subclass phenotype A (predominance of larger LDL subclass with peak diameter usually above 25.5 nm) and LDL phenotype B (predominance of smaller LDL subclass peak diameter below 25.5 nm) as described by Austin et al.

Electrophoresis of HDL in the ultracentrifuged d≤1.20 g/ml fraction was done on a Pharmacia Electrophoresis Apparatus (GE 4-II, Pharmacia, Piscataway, N.J.), using slab gradient gels (PAA 4/30, Pharmacia) as described by Blanche et al. The protein-stained gradient gels were scanned with a model RFT densitometer (Transidyne Corp., Ann Arbor, Mich.) at a wavelength of 603 nm. A mixture of four globular proteins (high-molecular-weight calibration kit) was run on the central lane to calibrate for particle size. The HDL migration distances (R₁) were measured relative to the migration distance of the peak of bovine serum albumin, one of the protein standards. The HDL distributions were converted from the migration distance scale to the particle diameter scale using a method previously described.

**Results**

Analytical ultracentrifugation was used to measure concentrations of total lipoprotein mass for 15 HDL flotation intervals between F₁0₂0-9, 11 LDL flotation intervals between S₁0-12, four intermediate density lipoprotein (IDL) flotation intervals between S₁2-20, and 14 very low density lipoprotein (VLDL) flotation intervals between S₂0-400. Results are presented for HDL₁ (F₁0₂5.5-9), HDL₂ (F₁0₂0-3.5), small LDL (S₀-7), large LDL (S₇-12), IDL (S₁₂-20), and VLDL (S₂₀-400) mass concentrations and for LDL peak flotation (S₁) rates (i.e., the mode of the LDL mass distribution).

**Statistical Analysis**

Conversion from absorbance to plasma concentration is not necessary for analyzing protein-stained HDL levels. The statistical tests used in this report (i.e., t tests, Pearson's correlation coefficients) are invariant to translations of scale or location. This means that the statistics and significance levels for absorbance will be identical to those based on unknown plasma concentrations when the conversion involves the addition and/or multiplication of numerical constants. In fact, different constants may be used at each diameter so that variation in chromogenicity across the HDL particle size spectrum will not affect the results. The two-tailed significance levels for t tests and Pearson's correlation coefficients presented in the figures and tables were verified by Spearman's correlations and Wilcoxon two-sample sign-rank tests. Nonparametric statistical tests of the protein-stained absorbance will be identical to those based on the unknown plasma concentrations, provided that ordering the gels from lowest to highest absorbance is the same as ordering the gels from lowest to highest plasma concentrations (i.e., monotonic relation). All significance levels are two tailed. A probability level of p≤0.01 was considered statistically significant.

**Results**

Table 1 presents the characteristics of the men and women. Figure 1 (top) displays the mean absorbance of protein-stained HDL by particle size. The difference between the men's and women's mean distribution is also displayed (bottom). The diameter values that achieved statistical significance (p<0.01) for the difference (two-sample t test) are designated by the solid portions of the bar at the bottom of the difference plot. Compared with women, men had significantly higher values for HDL between 7.4 and 8.3 nm (predominantly HDL₁₂ and HDL₁₃) and significantly lower values between 8.6- and 12-nm diameter (including HDL₂₀, HDL₂₁, and some HDL₁₃). The men were further divided on the basis of their LDL phenotype pattern. Thirty-nine men had the LDL subclass phenotype B, and 76 men had phenotype A. Figure 2 shows that the phenotype B men had significantly higher values between 7.7 and 8.1 nm (predominantly HDL₁₃) and significantly lower values between 8.6 and 11.9 nm (HDL₂₀, HDL₂₁, and some HDL₁₃). There were only four phenotype B women.

**Relations With High Density Lipoprotein Particle Size Distribution**

To better understand the relation of HDL subclasses with other lipoproteins, we first considered the interre-
TABLE 1. Lipoprotein and Other Characteristics of a Cross-Sectional Sample of Moderately Overweight Men and Women

<table>
<thead>
<tr>
<th>Sample divided by sex</th>
<th>All women (n=78)</th>
<th>All men (n=116)</th>
<th>Men divided by predominant peak</th>
<th>3a Peak (n=87)</th>
<th>3b Peak (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>38.4±6.5</td>
<td>40.2±6.4</td>
<td>39.9±6.6</td>
<td>41.3±5.7</td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>35.9±4.7</td>
<td>28.0±4.5*</td>
<td>27.7±4.4</td>
<td>28.7±4.7</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>193.6±29.7</td>
<td>210.1±33.6*</td>
<td>210.5±34.2</td>
<td>208.9±31.0</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>73.7±40.8</td>
<td>127.2±72.2*</td>
<td>109.0±55.7</td>
<td>182.0±88.0†</td>
<td></td>
</tr>
<tr>
<td>HDL&lt;sub&gt;2&lt;/sub&gt; cholesterol (mg/dl)</td>
<td>21.1±12.3</td>
<td>7.9±8.1*</td>
<td>9.9±8.1</td>
<td>1.9±4.1†</td>
<td></td>
</tr>
<tr>
<td>HDL&lt;sub&gt;3&lt;/sub&gt; cholesterol (mg/dl)</td>
<td>37.8±7.5</td>
<td>35.0±6.6</td>
<td>36.7±6.3</td>
<td>30.1±4.4†</td>
<td></td>
</tr>
<tr>
<td>HDL&lt;sub&gt;2&lt;/sub&gt; mass (mg/dl)</td>
<td>81.2±50.6</td>
<td>23.1±23.9*</td>
<td>29.0±24.9</td>
<td>5.6±4.4</td>
<td></td>
</tr>
<tr>
<td>HDL&lt;sub&gt;3&lt;/sub&gt; mass (mg/dl)</td>
<td>212.4±32.9</td>
<td>192.1±35.9*</td>
<td>202.3±32.9</td>
<td>161.5±26.3†</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/dl)</td>
<td>138.1±27.0</td>
<td>122.8±20.6*</td>
<td>128.8±18.9</td>
<td>105.2±14.7†</td>
<td></td>
</tr>
<tr>
<td>Small-LDL mass (mg/dl)</td>
<td>134.5±49.9</td>
<td>198.1±68.2*</td>
<td>184.7±66.4</td>
<td>238.3±57.8†</td>
<td></td>
</tr>
<tr>
<td>Large-LDL mass (mg/dl)</td>
<td>132.8±38.9</td>
<td>111.8±40.5*</td>
<td>124.2±38.0</td>
<td>74.7±19.9†</td>
<td></td>
</tr>
<tr>
<td>IDL mass (mg/dl)</td>
<td>18.7±15.5</td>
<td>30.6±18.0*</td>
<td>29.6±19.0</td>
<td>33.8±14.6</td>
<td></td>
</tr>
<tr>
<td>VLDL mass (mg/dl)</td>
<td>47.8±47.0</td>
<td>106.1±74.3*</td>
<td>87.0±61.5</td>
<td>163.1±81.0†</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dl)</td>
<td>69.9±16.4</td>
<td>86.7±19.9*</td>
<td>84.1±19.5</td>
<td>94.4±19.4†</td>
<td></td>
</tr>
<tr>
<td>LDL peak flotation rate (S&lt;sub&gt;p&lt;/sub&gt;)</td>
<td>7.1±1.2</td>
<td>5.9±1.3*</td>
<td>6.2±1.1</td>
<td>4.6±0.9†</td>
<td></td>
</tr>
<tr>
<td>LDL peak particle diameter (nm)</td>
<td>26.8±9.9</td>
<td>26.0±1.1*</td>
<td>26.4±1.0</td>
<td>25.1±0.8‡</td>
<td></td>
</tr>
<tr>
<td>Phenotype B (%)</td>
<td>5.0</td>
<td>33.9*</td>
<td>20.9</td>
<td>72.4†</td>
<td></td>
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</tbody>
</table>

Values are mean±SD. HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein. Significant difference between men and women at p<0.01. Significant difference between men with predominant 3a peak and 3b peak at p<0.01.

Lipid concentrations of HDL subclasses among themselves. These are displayed in the contour plots of Figure 3. The contours show the intercorrelations for protein-stained HDL at different diameter values in men (above the diagonal) and women (below the diagonal). Along the diagonal are the correlations of HDL at the same diameter values, which are all equal to 1. Each contour line represents an increment in the correlation of 0.1 unit. The plots for men and women show two similar features: 1) protein-stained HDL at all diameters greater than 8.3 nm are

![Figure 1. Plots showing mean absorbances of protein-stained high density lipoprotein (HDL) by particle size in men and women (upper panel) and their difference (lower panel). Solid portions of the bar at the bottom of the figure designate the diameter values that achieved statistical significance (p<0.01) for two-sample t test.](http://atvb.ahajournals.org/)
FIGURE 3. Contour plot of the intercorrelation of protein-stained high density lipoprotein (HDL) between 7.2 and 12 nm. Contours represent regions of equivalent correlation and are plotted every 0.1 unit change. The diagonal has a correlation of $r=1.0$, and solid and dashed lines represent positive and negative correlations, respectively. Shading designates significance at $p<0.01$.

positively correlated with one another (the lightly shaded areas), and 2) HDL$_{2a}$ is negatively correlated with HDL$_{3a}$ and HDL$_{2b}$ (darkly shaded areas).

Figure 4 displays the Pearson's correlation coefficients (vertical axis) for protein-stained absorbance at each HDL diameter value (horizontal axis) with selected lipoprotein measurements. The correlations for men and women are plotted separately. The correlations that achieved statistical significance (i.e., $r>0$) at $p<0.01$ are designated by the solid portions of the bar at the bottom of each graph. Some graphs are not displayed because of their similarity with other variables:

FIGURE 4. Plots showing Pearson correlation coefficients between levels of high density lipoprotein (HDL) protein and selected plasma lipoprotein measurements in 116 men and 78 women. Solid portions of the bars at the bottom of the graphs designate the range of diameter values that correlate significantly at $p<0.01$ or less. LDL, low density lipoprotein; IDL, intermediate density lipoprotein.
of these measurements. In men, high HDL sub cholesterol concentrations were associated with low triglyceride \((r=-0.31, p=0.001)\), low VLDL mass \((r=-0.26, p=0.005)\), and low small-LDL mass concentrations \((r=-0.37, p=0.0001)\) but not high triglycerides, VLDL mass, and small LDL as observed for HDL sub. Table 1 shows that HDL cholesterol is slightly higher in women than in men (opposite that observed for HDL sub).

Figure 3 suggests that HDL sub could be related to high-risk lipoproteins through its inverse correlation with HDL sub or HDL sub. This possibility was examined in Table 3 by partial correlation analysis. Absorbance at 8 nm was selected to represent HDL sub because it falls in the middle of the HDL sub range as defined by Blanche et al. Absorbance at the 10.5-nm diameter was selected to represent HDL sub. When adjusted for HDL sub, HDL sub protein retained its significant correlation with triglycerides, small LDL, VLDL mass, LDL peak flotation rate, and LDL peak particle diameter in men and women. These correlations were also significant when adjusted for HDL sub (represented by 9.3 nm; analyses not displayed).

**Relations With High Density Lipoprotein—Predominant Peak Diameter**

Prior studies have principally focused on the position of the HDL peak to characterize electrophoretic HDL distributions. The analyses to follow examine the relations of the peak diameter to other lipoprotein measurements and test whether the relations between lipoprotein measurements and HDL subclasses are adequately summarized by the peak diameter alone.

Significant lipoprotein differences are obtained when the men are divided according to whether their HDL peak diameter fell into the HDL sub or the HDL sub interval (Table 1). The 29 men whose predominant peak diameter fell within the 3b interval had significantly lower HDL mass (both HDL sub and HDL sub), apo A-I, LDL peak flotation rate, and LDL particle diameter and significantly higher triglycerides, small-LDL mass, VLDL mass, and apo B than the 87 men with a predominant HDL sub peak. Nearly three fourths of the men who had a predominant HDL sub peak were classified as LDL subclass phenotype pattern B. None of the women had their predominant HDL peak in the HDL sub interval.

Significant lipoprotein relations are also obtained when the diameter of the HDL peak is directly correlated with plasma lipoprotein measurements (Table 3). In both sexes, the predominant peak diameter correlates positively with HDL sub cholesterol, HDL sub mass, apo A-I, large-LDL mass, LDL peak flotation rate, and LDL peak particle diameter and negatively with small-LDL and VLDL concentrations. In men, predominant peak diameter correlates positively with HDL sub cholesterol and HDL sub mass and negatively with triglyceride and apo B concentrations.

Partial correlations (Table 3) were examined to test whether measurements of the predominant peak diameter were sufficient for describing the relations of HDL sub with other lipoproteins. A nonsignificant partial correlation shows that the absorbance within the HDL sub range is most important when examining lipoprotein correlations in women and in men with a predominant HDL sub peak in the HDL sub region. In men, lipid, lipoprotein, and apolipoprotein concentrations are usually correlated less strongly with protein-stained HDL at 8 nm than with predominant peak diameter. Adjustment for predominant peak diameter frequently eliminates the significance of the correlations between protein-stained HDL at 8 nm and the lipoprotein concentrations. Adjustment for predominant peak diameter was less likely to eliminate significant relations involving protein-

### Table 2. Summary of Relations of Protein-Stained High Density Lipoprotein to Plasma Lipid, Lipoprotein, and Apolipoprotein Levels in a Cross-Sectional Sample of 116 Men and 78 Women

<table>
<thead>
<tr>
<th>Apolipoprotein A-I</th>
<th>HDL sub</th>
<th>HDL sub</th>
<th>HDL sub</th>
<th>HDL sub</th>
<th>HDL sub</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0/0)</td>
<td>(0/0)</td>
<td>(+/+)</td>
<td>(+/+)</td>
<td>(+/+)</td>
<td>(+/+)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>(0/-)</td>
<td>(0/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>(0/0)</td>
<td>(-/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
</tr>
<tr>
<td>HDL3 cholesterol</td>
<td>(0/0)</td>
<td>(0/0)</td>
<td>(+/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
</tr>
<tr>
<td>HDL2 mass</td>
<td>(0/0)</td>
<td>(-/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
</tr>
<tr>
<td>HDL1 mass</td>
<td>(0/0)</td>
<td>(0/0)</td>
<td>(+/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
</tr>
<tr>
<td>LDL peak particle diameter</td>
<td>(-/-)</td>
<td>(-/-)</td>
<td>(0/0)</td>
<td>(+/-)</td>
<td>(+/-)</td>
</tr>
<tr>
<td>LDL peak flotation rate</td>
<td>(-/-)</td>
<td>(-/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
</tr>
<tr>
<td>Small LDL</td>
<td>(+/-)</td>
<td>(+/-)</td>
<td>(0/0)</td>
<td>(-/-)</td>
<td>(-/-)</td>
</tr>
<tr>
<td>Large LDL</td>
<td>(0/0)</td>
<td>(-/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
<td>(+/-)</td>
</tr>
<tr>
<td>IDL mass</td>
<td>(0/0)</td>
<td>(+/-)</td>
<td>(0/0)</td>
<td>(-/-)</td>
<td>(-/-)</td>
</tr>
<tr>
<td>VLDL mass</td>
<td>(0/0)</td>
<td>(+/-)</td>
<td>(0/0)</td>
<td>(-/-)</td>
<td>(-/-)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>(0/0)</td>
<td>(+/-)</td>
<td>(0/0)</td>
<td>(-/-)</td>
<td>(-/-)</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>(0/0)</td>
<td>(+/-)</td>
<td>(0/0)</td>
<td>(-/-)</td>
<td>(-/-)</td>
</tr>
</tbody>
</table>

HDL sub, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein.

**+** Designates a significant positive correlation \((p<0.01)\) for at least one half of the HDL subclass; "-" designates a significant negative correlation \((p<0.01)\) for at least one half of the subclass; "0" designates that the correlations are not significant for at least one half of the subclass.

### Figure 3

![Figure 3](https://example.com/figure3.png)

Table 3 suggests that HDL sub could be related to high-risk lipoproteins through its inverse correlation with HDL sub or HDL sub. This possibility was examined in Table 3 by partial correlation analysis. Absorbance at 8 nm was selected to represent HDL sub because it falls in the middle of the HDL sub range as defined by Blanche et al. Absorbance at the 10.5-nm diameter was selected to represent HDL sub. When adjusted for HDL sub, HDL sub protein retained its significant correlation with triglycerides, small LDL, VLDL mass, LDL peak flotation rate, and LDL peak particle diameter in men and women. These correlations were also significant when adjusted for HDL sub (represented by 9.3 nm; analyses not displayed).

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stained HDL at 8 nm in men with a predominant 3a peak. In contrast to the men, women's lipid, lipoprotein, and apolipoprotein concentrations are often correlated more strongly with protein-stained HDL at 8 nm than with predominant peak diameter.

Discussion

In their 1975 article, Miller and Miller, summarized the data suggesting that high HDL cholesterol levels were associated with low coronary heart disease risk. Attention then focused on the HDL subfractions identified by Lindgren et al in 1951. These included two lipoproteins of approximate hydrated densities of 1.075 and 1.12 g/ml that were labeled HDL2 and HDL3, respectively, by DeLalla and Gofman. Although exceptions exist, most studies show that high HDL2 levels are associated with reduced coronary heart disease risk.

Krauss et al found that HDL3 mass concentrations of F140–1.5 were higher in men than in women, were positively correlated with small LDL, and were negatively correlated with large LDL. These relations were also observed in the current sample (see “Appendix”). Unlike the gradient gel profiles of HDL protein, analytical ultracentrifuge showed no distinct HDL3, peak. The potential for small LDL (i.e., F140–1.5) to predict coronary heart disease may have been deemphasized because it appeared as a poorly resolved part of the HDL3 continuum instead of as a distinct HDL component.

Our results are consistent with the case–control study of Wilson et al. They compared the percentages of the total HDL that fell into each of the five subclasses in coronary survivors and controls. Compared with controls, the coronary survivors had less of their total HDL as HDL2 and more of their total as HDL3, HDL2, and HDL3. In their analyses, converting the heights of the curves into percentages necessarily induces a strong inverse correlation between the HDL2 and HDL3 subclasses. Our analyses suggest that the areas of the HDL subclasses can be used directly as an index of the mass concentration (i.e., without conversion into percentages). When this is done, HDL2 is associated with a profile of lipoproteins suggestive of low coronary heart disease risk, and HDL3 is associated with a profile suggestive of increased risk, independent of HDL2.

Table 3. Pearson Correlation Coefficients for High Density Lipoprotein–Predominant Peak Diameter and Absorbance of High Density Lipoprotein Protein at 8 nm (Within High Density Lipoprotein Subclasses) and Plasma Lipids, Lipoproteins, and Apolipoproteins

<table>
<thead>
<tr>
<th>Correlation with HDL-predominant peak diameter</th>
<th>Correlation with absorbance of HDL protein at 8 nm</th>
<th>All women</th>
<th>All men</th>
<th>Men with predominant HDL, peak</th>
<th>All women</th>
<th>All men</th>
<th>Men with predominant HDL, peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td></td>
<td>0.04</td>
<td>-0.06</td>
<td>-0.29*</td>
<td>0.19</td>
<td>0.27*†‡</td>
<td>0.45**†‡</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td>-0.25</td>
<td>-0.49*</td>
<td>-0.41*</td>
<td>0.59†‡</td>
<td>0.38‡†</td>
<td>0.51**†‡</td>
</tr>
<tr>
<td>HDL2 cholesterol</td>
<td></td>
<td>0.49*</td>
<td>0.49*</td>
<td>0.28*</td>
<td>-0.49*</td>
<td>-0.26*</td>
<td>-0.21</td>
</tr>
<tr>
<td>HDL2 cholesterol</td>
<td></td>
<td>0.23</td>
<td>0.45*</td>
<td>0.17</td>
<td>-0.21</td>
<td>-0.07</td>
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</tr>
<tr>
<td>HDL2 mass</td>
<td></td>
<td>0.53*</td>
<td>0.54*</td>
<td>0.46*</td>
<td>-0.57†‡</td>
<td>-0.34†‡</td>
<td>-0.26‡</td>
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<td>0.48*</td>
<td>0.05</td>
<td>0.09</td>
<td>0.12†‡</td>
<td>0.34**†‡</td>
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<tr>
<td>Apolipoprotein A-I</td>
<td></td>
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<td>0.50*</td>
<td>0.16</td>
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<td>-0.02†‡</td>
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<tr>
<td>Small-LDL mass</td>
<td></td>
<td>-0.40*</td>
<td>-0.45*</td>
<td>-0.44*</td>
<td>0.56†‡</td>
<td>0.40*‡</td>
<td>0.47†‡</td>
</tr>
<tr>
<td>Large-LDL mass</td>
<td></td>
<td>0.31*</td>
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<tr>
<td>IDL mass</td>
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<td>-0.14</td>
<td>-0.21</td>
<td>-0.37</td>
<td>0.40†‡</td>
<td>0.31†‡</td>
<td>0.43†‡</td>
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<tr>
<td>VLDL mass</td>
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<td>-0.31*</td>
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<td>-0.39*</td>
<td>0.55†‡</td>
<td>0.40*‡</td>
<td>0.49†‡</td>
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<tr>
<td>Apolipoprotein B</td>
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<td>-0.25</td>
<td>-0.31*</td>
<td>-0.37</td>
<td>0.46†‡</td>
<td>0.29*</td>
<td>0.44†‡</td>
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<tr>
<td>LDL peak flotation rate</td>
<td></td>
<td>0.40*</td>
<td>0.62*</td>
<td>0.45*</td>
<td>-0.65†‡</td>
<td>-0.45†‡</td>
<td>-0.44‡</td>
</tr>
<tr>
<td>LDL peak particle diameter</td>
<td></td>
<td>0.32*</td>
<td>0.61*</td>
<td>0.50*</td>
<td>-0.56†‡</td>
<td>-0.54†‡</td>
<td>-0.55**†‡</td>
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</tbody>
</table>

*Significantly different from zero at p<0.01.
†Significantly different from zero at p<0.01 when adjusted for peak diameter.
‡Significantly different from zero at p<0.01 when adjusted for protein-stained HDL at 10.5 nm (within HDL2).

Our results are consistent with the case–control study of Wilson et al. They compared the percentages of the total HDL that fell into each of the five subclasses in coronary survivors and controls. Compared with controls, the coronary survivors had less of their total HDL as HDL2 and more of their total as HDL3, HDL2, and HDL3. In their analyses, converting the heights of the curves into percentages necessarily induces a strong inverse correlation between the HDL2 and HDL3 subclasses. Our analyses suggest that the areas of the HDL subclasses can be used directly as an index of the mass concentration (i.e., without conversion into percentages). When this is done, HDL2 is associated with a profile of lipoproteins suggestive of low coronary heart disease risk, and HDL3 is associated with a profile suggestive of increased risk, independent of HDL2.
FIGURE 5. Plots showing Pearson correlation coefficients between analytical ultracentrifuge measurements of high density lipoprotein$_{a_1}$ mass by individual flotation intervals in 116 men and 78 women. Distance between each horizontal axis (dashed line) covers 0 to +1 or 0 to −1. Solid portions of the bars at the bottom of the graphs designate statistical significance at $p \leq 0.01$.

production of small LDL so that small LDL and HDL$_{a_1}$ increase in parallel. The majority of men having a predominant HDL$_{a_1}$ peak were classified as LDL subclass phenotype pattern B. Austin et al.\(^3\) report that this phenotype is inherited through a dominant allele, with a frequency of 25%. Therefore, HDL$_{a_1}$ values might also segregate in families if the genetic determinants of LDL phenotype B also affect HDL$_{a_1}$ concentrations.

The analysis of the total protein-stained HDL profile is a sensitive and practical means of assessing the HDL particle size distribution. The "Appendix" shows that the correlations for protein-stained HDL are comparable to those obtained for analytical ultracentrifuge measurements of plasma HDL mass concentrations. Previous electrophoretic studies have used the position of the major peak and the statistical estimation of components by deconvolution to interpret their results. The usefulness of predominant HDL peak diameter is evident from Tables 1 and 3. Yet, Table 3 also shows that this measurement discards important information contained in the absorbance of the protein-stained HDL. When the technique of transformation of variables is used to correctly estimate the predominant HDL peak diameter, there is little additional effort required for determining the total HDL profile.\(^{14}\) Statistical estimation of HDL components by deconvolution may be misleading when the analyses use the wrong distributions. For example, Verdery et al.\(^7\) found no significant relation between HDL$_{a_1}$ and plasma triglyceride levels. The particle distribution in some or all of the HDL subfractions might not be normally distributed so that HDL$_{a_1}$ levels might not be correctly estimated by Gaussian summation.

Reverse transport of cholesterol from tissues to the liver for excretion via HDL has been postulated to explain the putative protective effects of HDL cholesterol in coronary disease risk.\(^{32}\) Yet, high HDL cholesterol concentrations appear to result from the accumulation of cholesterol in HDL, and this may not be indicative of increased reverse cholesterol transport. Figures 3 and 4 suggest that the protective effects of high HDL cholesterol or HDL$_{a_1}$ concentrations might be due in part to associations with low HDL$_{a_1}$ values. Nothing is known about HDL$_{a_1}$ and coronary heart disease risk from prospective epidemiological trials or case-control studies. Previous studies measured HDL$_1$ cholesterol or HDL$_3$ mass, which appear to be poor indicators of HDL$_{a_1}$ levels. Figure 4 shows that HDL$_1$ cholesterol primarily reflects variations in protein-stained HDL$_{a_1}$ and HDL$_{a_2}$ on polyacrylamide gradient gels and shows no relation to HDL$_{a_1}$ levels. For this reason, studies showing concordant,\(^3\) discordant,\(^3\) or no\(^3\)–\(^7\) relation between coronary heart disease and HDL$_1$ cholesterol or mass probably bear little relevance to HDL$_{a_1}$.

Appendix

Relation Between Analytical Ultracentrifugation and Gradient Gel Electrophoresis

This appendix examines the correlations between HDL protein from gradient gel electrophoresis and HDL mass from analytical ultracentrifugation. Our objective is to identify the flotation intervals that correspond to the HDL$_{a_1}$ component. We also examine the correlations between HDL mass and other lipoproteins to compare them with those obtained for HDL absorbance.

Figure 5 shows the correlations of HDL protein versus HDL total mass for the seven individual flotation intervals within the HDL$_1$ range of the analytical ultracentrifuge. HDL$_{a_1}$ protein correlated positively with HDL mass of F$_{1,0}$ to 1.0 in men and of F$_{1,0}$ to 1.5 in women. Although the next lightest flotation
when lipoprotein and apolipoprotein concentrations were comparable correlation coefficients were obtained with HDL mass concentrations (Table 4) as with protein-VLDL mass in both men and women; 2) F_{upO-1.0} mass is mass versus HDL 2 and HDL 3 cholesterol overlap extensively. In stained HDL (Figure 4). Specifically, 1) low-buoyancy HDL lipoprotein concentrations generally exhibit the same relations HDL*. Table 4 displays the correlations for analytical ultracentrifugation measurements of HDL mass by flotation rate versus HDL 2 cholesterol and positively with triglycerides, apo B, IDL mass, and triglyceride positively with HDL they also correlate positively with

<table>
<thead>
<tr>
<th></th>
<th>Apo</th>
<th>HDL</th>
<th>HDL 2</th>
<th>HDL 3</th>
<th>Triglycerides</th>
<th>Apo B</th>
<th>IDL</th>
<th>VLDL</th>
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<td>cholesterol</td>
<td>cholesterol</td>
<td>Triglycerides</td>
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<td>0.23</td>
<td>0.00</td>
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<td>0.16</td>
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<td>0.31*</td>
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<td>0.69*</td>
<td>0.51*</td>
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<td>-0.27*</td>
<td>-0.18</td>
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<td>-0.40*</td>
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<td>0.04</td>
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<td>-0.16</td>
</tr>
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</table>

Apo, apolipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein.
*Correlation significantly different from zero at \( p < 0.01 \).

intervals (\( F_{1,30} \) 0.0-1.5 in men and \( F_{1,30} \) 1.5-2.0 in women) correlate positively with HDL, they also correlate positively with HDL. Table 4 displays the correlations for analytical ultracentrifugation measurements of HDL mass by flotation rate versus lipoproteins and apolipoproteins. Plasma lipoprotein and apolipoprotein concentrations generally exhibit the same relations with HDL mass concentrations (Table 4) as with protein-stained HDL (Figure 4). Specifically, 1) low-buoyancy HDL particles (\( F_{1,30} \) 1.5) correlated negatively with HDL cholesterol and positively with triglycerides, apo B, IDL mass, and VLDL mass in both men and women; 2) \( F_{1,30} \) 0.0-1.0 mass is unrelated to HDL cholesterol; and 3) the correlations of HDL mass versus HDL and HDL cholesterol overlap extensively. In general, comparable correlation coefficients were obtained when lipoprotein and apolipoprotein concentrations were correlated with electrophoresis protein-stained HDL (Figure 4) and analytical ultracentrifuge HDL mass (Table 4).

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