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

Paul T. Williams, Ronald M. Krauss, Karen M. Vranizan, Marcia L. Stefanick, Peter D.S. Wood, and Frank T. Lindgren

With the technical assistance of Laura Holl, Charlotte Brown, and Bahareh Sahami

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_1 and significantly lower HDL_2 and HDL_3 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_1 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_3 mass. Plasma concentrations of HDL_3 cholesterol were unrelated to protein-stained HDL_2 levels. HDL_3 cholesterol concentrations also did not exhibit the sex difference or the relations with lipoprotein concentrations that characterized HDL_1. Thus, low HDL_2 levels may contribute in part to the low heart disease risk in men and women who have high HDL cholesterol. Measurements of HDL_3 cholesterol may not identify clinically important relations involving HDL_1. (Arteriosclerosis and Thrombosis 1992;12:332-340)

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 nondenaturing 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 peaks1-5; 2) the proportion of HDL protein occurring within intervals believed to contain predominantly HDL subclasses; and 3) component distributions that are assumed to have normal (Gaussian) shapes.7

In this report, we examine the relations of gender, plasma lipoproteins, and apolipoproteins to protein-stained HDLs 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 electro-
E.20 HDL distributions were converted from the migration (R,)
distance scale to the particle diameter scale using a serum albumin, one of the protein standards. The relative to the migration distance of the peak of bovine stained gradient gels were scanned with a model RFT 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.),8-9 and HDL cholesterol was measured by dextran sulfate–magnesium precipitation method, and LDL cholesterol was calculated as the difference between total HDL cholesterol and HDL cholesterol.10 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 and cholesterol was determined by a dextran sulfate–magnesium precipitation method, and LDL cholesterol was calculated as the difference between total HDL cholesterol and HDL cholesterol.11 Plasma apolipoprotein (apo) A-I and B concentrations were determined by rate immunonephelometry (Beckman ARRAY, Beckman Instruments, Palo Alto, Calif.).12 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 of peak diameter below 25.5 nm) as described by Austin et al.13 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.1 The protein-stained gradient gels were scanned with a model RFT densitometer (Transdyne 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 (R,0) 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.14

Analytical ultracentrifugation was used to measure concentrations of total lipoprotein mass for 15 HDL flotation intervals between F,0-9, 11 LDL flotation intervals between S,0-12, four intermediate density lipoprotein (IDL) flotation intervals between S,12-20, and 14 very low density lipoprotein (VLDL) flotation intervals between S,20-400.15 Results are presented for HDL (F,3.5-9), HDL (F,3.5-3.5), small LDL (S,0-7), large LDL (S,7-12), IDL (S,12-20), and VLDL (S,20-400) mass concentrations and for LDL peak flotation (S,0) 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 HDL and HDL) and significantly lower values between 8.6- and 12-nm diameter (including HDL, HDL, and some HDL). 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 HDL) and significantly lower values between 8.6 and 11.9 nm (HDL, HDL, and some HDL). 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>Men divided by predominant peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All women (n=78)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>38.4±6.5</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>35.9±4.7</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>193.6±29.7</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>73.7±40.8</td>
</tr>
<tr>
<td>HDL₂ cholesterol (mg/dl)</td>
<td>21.1±12.3</td>
</tr>
<tr>
<td>HDL₃ cholesterol (mg/dl)</td>
<td>37.8±7.5</td>
</tr>
<tr>
<td>HDL₂ mass (mg/dl)</td>
<td>81.2±50.6</td>
</tr>
<tr>
<td>HDL₃ mass (mg/dl)</td>
<td>212.4±32.9</td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/dl)</td>
<td>138.1±27.0</td>
</tr>
<tr>
<td>Small-LDL mass (mg/dl)</td>
<td>134.5±49.9</td>
</tr>
<tr>
<td>Large-LDL mass (mg/dl)</td>
<td>132.8±38.9</td>
</tr>
<tr>
<td>IDL mass (mg/dl)</td>
<td>18.7±15.5</td>
</tr>
<tr>
<td>VLDL mass (mg/dl)</td>
<td>47.8±47.0</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dl)</td>
<td>69.9±16.4</td>
</tr>
<tr>
<td>LDL peak flotation rate (Sₖ)</td>
<td>7.1±1.2</td>
</tr>
<tr>
<td>LDL peak particle diameter (nm)</td>
<td>26.8±9.9</td>
</tr>
<tr>
<td>Phenotype B (%)</td>
<td>5.0</td>
</tr>
</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.

The correlations 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](http://atvb.ahajournals.org/)

**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.

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Plots showing mean absorbances of protein-stained high density lipoprotein (HDL) by particle size in 39 men classified as low density lipoprotein (LDL) phenotype pattern B and 76 men classified as LDL phenotype A (upper panel) and their difference (lower panel). Solid portions of the bar designate the diameter values that achieved statistical significance (p<0.01) for two-sample t test.
positively correlated with one another (the lightly shaded areas), and 2) HDL_{3a} is negatively correlated with HDL_{3c} and HDL_{3b} (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: i.e., HDL_{2c} mass with HDL_{2a} cholesterol, HDL_{3a} mass with HDL_{3c} cholesterol, VLDL mass and apo B with triglycerides, and LDL peak particle diameter with LDL peak flotation rate. Table 2 summarizes these relations.

Figure 4 and Table 2 suggest that the correlations fall into two basic patterns: 1) HDL_{2c} mass, HDL_{2a} cholesterol, LDL peak flotation rate, LDL peak particle diameter, and large-LDL mass (men) all correlate negatively with HDL_{3c} and positively with HDL_{3a} and HDL_{2a} and 2) small LDL, VLDL mass, triglycerides, apo B, and IDL mass (men) all correlate positively with HDL_{3c} and negatively with HDL_{3b} and HDL_{2a}. These patterns reflect the inverse correlations of HDL_{3c} with HDL_{2a} and HDL_{2b} among HDL subclasses (Figure 3).

Figure 4 and Table 2 also suggest there are sex differences in the correlations. Many of the lipoproteins associated with low coronary heart disease risk (i.e., HDL_{3c}, large LDL, and LDL peak flotation rate) correlate positively with HDL_{3a} in men but not in women. This is only partially explained by the larger sample size for men. Specifically, HDL_{3b} correlates significantly more strongly with HDL cholesterol, HDL_{3a} cholesterol, and LDL peak flotation rate in men than in women for >85% of the HDL_{3c} particle range.

Finally, Figure 4 and Table 2 show that neither HDL_{3c} cholesterol nor HDL_{3b} mass is significantly correlated with HDL_{2a} protein. HDL_{3a} cholesterol correlates most strongly with HDL_{3c} protein. It is not surprising, therefore, that HDL_{3a} cholesterol exhibited none of the lipoprotein correlations nor sex differences that characterized HDL_{3c} protein. In women, HDL_{3a} cholesterol was unrelated to apo B, triglycerides, small-LDL mass, VLDL mass, and HDL_{2c} cholesterol (analyses not presented), whereas HDL_{3b} correlated significantly with all
of these measurements. In men, high HDL$_3$ 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$_{30}$. Table 1 shows that HDL$_3$ cholesterol is slightly higher in women than in men (opposite that observed for HDL$_{30}$. Figure 3 suggests that HDL$_{30}$ could be related to high-risk lipoproteins through its inverse correlation with HDL$_{30}$ or HDL$_{30}$. This possibility was examined in Table 3 by partial correlation analysis. Absorbance at 8 nm was selected to represent HDL$_{20}$ because it falls in the middle of the HDL$_{20}$ range as defined by Blanche et al.$^1$ Absorbance at the 10.5-nm diameter was selected to represent HDL$_{30}$. When adjusted for HDL$_{30}$, HDL$_{20}$ 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$_{30}$ (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.$^{1-5}$ 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 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$_2$ cholesterol, HDL$_2$ 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$_1$ cholesterol and HDL$_3$ 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$_{30}$ with other lipoproteins. A nonsignificant partial correlation shows that the absorbance within the HDL$_{30}$ range is redundant to the measurement of peak diameter, whereas a significant partial correlation shows that absorbance provides additional relevant information. The analysis of Table 3 suggests that HDL$_{30}$ absorbance is most important when examining lipoprotein correlations in women and in men with a predominant HDL$_{30}$ peak in the HDL$_{30}$, 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-
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. These included two lipoproteins of approximate hydrated densities of 1.075 and 1.12 g/ml that were labeled HDL. and HDL. respectively, by DeLalla and Gofman. Although exceptions exist, most studies show that high HDL levels are associated with reduced coronary heart disease risk. Less certain is the relation between HDL and coronary heart disease. Figures 1–4 present circumstantial evidence that high levels of one HDL component, HDL, might be associated with increased coronary heart disease risk. Specifically, we found that 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 HDL and more of their total as HDL and HDL. In their analyses, converting the heights of the curves into percentages necessarily induces a strong inverse correlation between the HDL and HDL 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, HDL is associated with a profile of lipoproteins suggestive of low coronary heart disease risk, and HDL is associated with a profile suggestive of increased risk, independent of HDL.

Krauss et al found that HDL mass concentrations of F, 0.5–1.5 were higher in men than in women, and 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 HDL. peak. The potential for small HDL (i.e., F, 0.5–1.5) to predict coronary heart disease may have been deemphasized because it appeared as a poorly resolved part of the HDL continuum instead of as a distinct HDL component.

The relations between triglycerides and protein-stained HDL shown in Figure 4 are consistent with in vitro experiments. High plasma triglyceride levels may promote HDL production through the sequential actions of cholesterol ester triglyceride exchange and HDL-triglyceride hydrolysis by hepatic lipase. Low triglyceride levels may reduce cholesterol ester--triglyceride exchange. This might promote the formation of HDL and HDL as cholesterol ester accumulates in HDL. Increased triglyceride levels may also promote

### 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All women</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.04</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.25</td>
</tr>
<tr>
<td>HDL, cholesterol</td>
<td>0.49*</td>
</tr>
<tr>
<td>HDL, cholesterol</td>
<td>0.23</td>
</tr>
<tr>
<td>HDL, mass</td>
<td>0.53*</td>
</tr>
<tr>
<td>HDL, mass</td>
<td>0.23</td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>0.32*</td>
</tr>
<tr>
<td>Small-LDL mass</td>
<td>-0.40*</td>
</tr>
<tr>
<td>Large-LDL mass</td>
<td>0.31*</td>
</tr>
<tr>
<td>IDL mass</td>
<td>-0.14</td>
</tr>
<tr>
<td>VLDL mass</td>
<td>-0.31*</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>-0.25</td>
</tr>
<tr>
<td>LDL peak flotation rate</td>
<td>0.40*</td>
</tr>
<tr>
<td>LDL peak particle diameter</td>
<td>0.32*</td>
</tr>
</tbody>
</table>

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

*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 8.5 nm (within HDL).
production of small LDL so that small LDL and HDL\textsubscript{3b} increase in parallel. The majority of men having a predominant HDL\textsubscript{3b} peak were classified as LDL sub-class phenotype pattern B. Austin et al\textsuperscript{31} report that this phenotype is inherited through a dominant allele, with a frequency of 25%. Therefore, HDL\textsubscript{3b} values might also segregate in families if the genetic determinants of LDL phenotype B also affect HDL\textsubscript{3b} 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.\textsuperscript{14} Statistical estimation of HDL components by deconvolution may be misleading when the analyses use the wrong distributions. For example, Verdery et al\textsuperscript{32} found no significant relation between HDL\textsubscript{3b} and plasma triglyceride levels. The particle distribution in some or all of the HDL subfractions might not be normally distributed so that HDL\textsubscript{3b} 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.\textsuperscript{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\textsubscript{3b} concentrations might be due in part to associations with low HDL\textsubscript{3b} values. Nothing is known about HDL\textsubscript{3b} and coronary heart disease risk from prospective epidemiological trials or case-control studies. Previous studies measured HDL\textsubscript{3} cholesterol or HDL\textsubscript{3} mass, which appear to be poor indicators of HDL\textsubscript{3b} levels. Figure 4 shows that HDL\textsubscript{3} cholesterol primarily reflects variations in protein-stained HDL\textsubscript{3b} and HDL\textsubscript{3a} on polyacrylamide gradient gels and shows no relation to HDL\textsubscript{3b} levels. For this reason, studies showing concordant,\textsuperscript{33} discordant,\textsuperscript{34} or no\textsuperscript{35-37} relation between coronary heart disease and HDL\textsubscript{3} cholesterol or mass probably bear little relevance to HDL\textsubscript{3b}.

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\textsubscript{3b} 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\textsubscript{3} range of the analytical ultracentrifuge. HDL\textsubscript{3b} protein correlated positively with HDL mass of F1\textsubscript{20} 0.5–1.0 in men and of F1\textsubscript{20} 1.0–1.5 in women. Although the next lightest flotation
intervals (F₁,₂₋₁.₅₀ in men and F₁,₂₋₁.₅₋₂.₀ in women) correlate positively with HDL₃, they also correlate positively with HDL₄. 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 (F₁,₂₋₁.₀₋₁.₅) correlated negatively with HDL₂ cholesterol and positively with triglycerides, apo B, IDL mass, and VLDL mass in both men and women; 2) F₁,₂₋₁.₀₋₁.₅ mass is comparable correlation coefficients were obtained with HDL mass versus HDL₂ and HDL₃ 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 ultracentrifugation HDL mass (Table 4).

References
6. Griffin BA, Skinner ER, Maughan RJ: Plasma high density lipoprotein subclasses in subjects with different coronary risk indices as assessed by plasma lipoprotein concentrations. Atherosclerosis 1988;70:165-169


11. Warnick GR, Bender A, Albers JJ: Quantification of high density lipoprotein subclasses after separation by dextran sulfate and Mg<sup>2+</sup> precipitation (abstract). Clin Chem 1982;28:1574


34. Wallentin L, Sundin B: HDL2 and HDL3 lipid levels in coronary artery disease. Atherosclerosis 1989;77:131–136


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