Distribution of ApoA-I–Containing HDL Subpopulations in Patients With Coronary Heart Disease

Bela F. Asztalos, Paul S. Roheim, Richard L. Milani, Michael Lefevre, Judith R. McNamara, Katalin V. Horvath, Ernst J. Schaefer

Abstract—High density lipoproteins (HDLs) and their subspecies play a role in the development of coronary heart disease (CHD). HDL subpopulations were measured by 2-dimensional nondenaturing gel electrophoresis in 79 male control subjects and 76 male CHD patients to test the hypothesis that greater differences in apolipoprotein (apo)A-I–containing HDL subpopulations would exist between these 2 groups than for traditional lipid levels. In CHD subjects, HDL cholesterol (HDL-C) was lower (−14%, P<0.001), whereas total cholesterol and the low density lipoprotein cholesterol/HDL-C ratio were higher (9% [P<0.05] and 21% [P<0.01], respectively) compared with control levels. No significant differences were found for low density lipoprotein cholesterol, triglyceride, and apoA-I levels. In CHD subjects, there were significantly (P<0.001) lower concentrations of the large lipoprotein (Lp)A-I α1 (−35%), pre-α1 (−50%), pre-α2 (−33%), and pre-α3 (−31%) subpopulations, whereas the concentrations of the small LpA-I/A-II α3 particles were significantly (P<0.001) higher (20%). Because α1 was decreased more than HDL-C and plasma apoA-I concentrations in CHD subjects, the ratios of HDL-C to α1 and of apoA-I to α1 were significantly (P<0.001) higher by 36% and 57%, respectively, compared with control values. Subjects with low HDL-C levels (≤35 mg/dL) have different distributions of apoA-I–containing HDL subpopulations than do subjects with normal HDL-C levels (>35 mg/dL). Therefore, we stratified participants according to HDL-C concentrations into low and normal groups. The differences in lipid levels between controls and HDL-C–matched cases substantially decreased; however, the significant differences in HDL subspecies remained. Our research findings support the concept that compared with control subjects, CHD patients not only have HDL deficiency but also have a major rearrangement in the HDL subpopulations with significantly lower α1 and pre-α1,3 (LpA-I) and significantly higher α3 (LpA-I/A-II) particles. (Arterioscler Thromb Vasc Biol. 2000;20:2670–2676.)

Key Words: HDL subpopulations • coronary heart disease • lipids • lipoproteins • apolipoproteins

Coronary heart disease (CHD) remains the leading cause of death and disability in the United States. Approximately 14 million people have CHD, and 1.5 million individuals experience a myocardial infarction annually, leading to 500 000 deaths per year.1 Atherosclerosis is a multifactorial disease affected by lifestyle and genetic factors.2 Independent risk factors for CHD as defined by the National Cholesterol Education Program (NCEP) Adult Treatment Panel II include the following: age, sex, hypertension, smoking, diabetes, family history of premature CHD, elevated plasma levels of LDL cholesterol (LDL-C ≥160 mg/dL), and low levels of HDL (HDL-C <35 mg/dL).3–10 The NCEP panel classified an HDL-C level ≥60 mg/dL (>1.55 mmol/L) as protective against the development of CHD.3 Many prospective epidemiological studies have indicated that a decreased HDL-C level is a significant independent risk factor for heart disease.5–7,9,11,12 HDL is found in human plasma at a density of 1.063 to 1.21 g/mL and contains ≈50% protein, 25% phospholipid, 20% cholesterol (mainly esterified), and 5% triglycerides by weight. The 2 major protein constituents are apoA-I and apoA-II. A variety of methods, including analytical ultracentrifugation, differential precipitation, immunoaffinity chromatography, and nondenaturing 1D and 2D gel electrophoresis, have been developed to separate HDL into different subclasses. These HDL subpopulations differ in apolipoprotein and lipid composition, as well as in size and charge, and probably have different physiological functions.13–16 Separation of HDL subclasses on the basis of charge and size by 2D nondenaturing gel electrophoresis was developed in the late 1980s14 and later was adopted by others.16–18 By use of this method, the majority of apoA-I in plasma has α mobility. These HDL particles have been classified as α1, α2, and α3, with sizes of 11.2, 9.51, and 7.12 nm, respectively.18

Received April 26, 2000; revision accepted September 21, 2000.
From the Lipid Metabolism Laboratory, Jean Mayer USDA Human Nutrition Center on Aging at Tufts University, and the Division of Endocrinology, Metabolism, Diabetes, and Molecular Medicine (B.F.A., J.R.M., K.V.H., E.J.S.), New England Medical Center, Boston, Mass; the Physiology Department (P.S.R.), Louisiana State University Medical Center, New Orleans; the Division of Cardiology (R.L.M.), Alton Ochsner Hospital, New Orleans, La; and Pennington Biomedical Research Center (M.L.), Baton Rouge, La.
Correspondence to Bela F. Asztalos, PhD, JM-USDA/HNRC at Tufts University, Lipid Metabolism Laboratory, 711 Washington St, Boston, MA 02111. E-mail belasztalos@yahoo.com
© 2000 American Heart Association, Inc.
Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org
Small amounts of HDL particles containing apoA-I with pre-β mobility (pre-β1 and pre-β2) have been detected as well. We have standardized the 2D nondenaturing gel electrophoresis methodology and have reported 12 apoA-I–containing HDL subpopulations, including 4 new subpopulations with pre-α mobility containing only apoA-I. apoA-II is present in α2 and α1 HDL subpopulations. Subjects with low HDL-C levels have significant decreases in the large LpA-I HDL particles, α1, and pre-α1.

The precise function and the metabolic nature of the apoA-I–containing and/or other apolipoprotein-containing HDL subfractions remain to be elucidated. There are a number of epidemiological studies on the relationship between CHD and HDL subspecies with conflicting results. Most investigators report an overall reduction in HDL-C and apoA-I with larger sample sizes and with more complete characterization of HDL subspecies. These issues require resolution between CHD patients and control individuals. They found that the presence of CHD was more strongly associated with HDL particle size distribution than with a low HDL-C level. The discrepancy between studies may be due to sample size and/or subject selection. Alternatively, the difference in results between studies may be explained by the fact that HDL2, HDL3, LpA-I, and LpA-I/A-II are heterogeneous fractions and contain a variety of different HDL subspecies. These issues require resolution with larger sample sizes and with more complete characterization of HDL subspecies.

Our goal was to determine and compare HDL subpopulations, as defined by 2D nondenaturing gel electrophoresis, in healthy male controls and male subjects with CHD. The present study provides additional information about the potential role of HDL subpopulations in the risk for CHD.

**Methods**

**Study Samples**
Fasting plasma samples were obtained from 79 healthy male subjects, aged 30 to 82 years. Participants were selected to have total plasma cholesterol and LDL-C levels between the 10th and 90th percentile, HDL-C levels >25 mg/dL or below the 95th percentile, and triglycerides below the 95th percentile. Lipid cutoff points were selected to eliminate subjects with lipid disorders. Exclusion criteria included the presence of cardiovascular, renal, hepatic, endocrine, gastrointestinal, or other systemic disease, body mass index >32 kg/m², and hypertension. During screening visits, physical examinations, blood chemistry, lipid profiles, and urinalyses were performed. Fasting plasma samples were also obtained from 76 male patients, aged 33 to 81 years, with established CHD, as documented by history of prior myocardial infarction, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, documented disease on coronary angiography (>50% stenosis in ≥1 coronary artery), or the presence of angina pectoris with a positive stress test. Exclusion criteria included triglyceride values >400 mg/dL, body mass index >35 kg/m², secondary causes of hyperlipoproteinemia, diabetes, any systemic disease interfering with lipoprotein metabolism, lipid-lowering medications, and/or myocardial infarction, and/or coronary artery bypass graft within the last 12 weeks.

**TABLE 1. ApoA-I–Containing HDL Subpopulations in Fresh and Stored Plasma Samples**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Distribution, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pre-β1</td>
<td>2.4 ± 1.2</td>
</tr>
<tr>
<td>Pre-β2</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>α2</td>
<td>18.4 ± 7.5</td>
</tr>
<tr>
<td>α3</td>
<td>39.0 ± 2.5</td>
</tr>
<tr>
<td>Pre-α1</td>
<td>30.9 ± 9.6</td>
</tr>
<tr>
<td>Pre-α2</td>
<td>2.5 ± 1.2</td>
</tr>
<tr>
<td>Pre-α3</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>Pre-α4</td>
<td>1.4 ± 0.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Blood samples (n=6) were collected in tubes containing EDTA (1.2 g/L), sodium azide (0.1 g/L), and aprotinin (10 kU/L) on ice. After slow-speed centrifugal separation of plasma, 1 mmol/L benzamidine and phenylmethylsulfonyl fluoride were added to the tubes. Samples are as follows: 1, Samples were immediately subjected to electrophoresis. 2, Aliquots were frozen at −80°C for 2 weeks before being subjected to electrophoresis. Before use, plasma samples were put into liquid nitrogen for at least 30 minutes before thawing in 37°C water bath. 3 and 4, Conditions were same as for sample 2, but plasma samples were stored for 2 months and 1 year, respectively, before use.

**Lipid and Lipoprotein Analysis**
Fasting blood samples were collected into tubes containing 1.5 g/L EDTA and centrifuged at 3000g for 20 minutes at 4°C to obtain plasma. Aliquots of each plasma sample were stored at −80°C or in liquid nitrogen until use. Total cholesterol, HDL-C, and triglycerides were determined by use of automated enzymatic assays. HDL-C was determined after precipitation of the non-HDL fraction by the dextran sulfate method. LDL-C was calculated by use of the Friedewald equation. apoA-I was measured in plasma by automated immunoturbidimetric assay (Wako Inc). Coefficients of variation for all assays between and within runs were <10%.

**HDL Subpopulation Analysis**
Separation of HDL subpopulations was carried out similarly as previously described in detail. Plasma samples stored at −80°C were placed into liquid nitrogen for ≈30 minutes before thawing at 37°C to avoid any alterations in the physicochemical composition of the particles. The effects of storage and thawing conditions were carefully investigated (Table 1). No significant differences in the percent distribution of the apoA-I–containing HDL subpopulations were detected (at 95% CI) when fresh plasma and stored plasma samples (up to 1 year) were compared.

In the first dimension, we separated HDL according to charge into pre-β, α1, and pre-α mobility particles. Four microliters of plasma was applied to 0.7% LE SeaKem agarose gels (3 mm thick) and electrophoresed in a vertical slab gel electrophoresis unit (Pharmacia GE 2/4 recirculating apparatus) in 25 mmol/L Tris-tricine buffer at pH 8.6 (10°C) at 250 V until the albumin (the blue band) reached 3.5 cm. Gel strips were cut out, placed, and sealed (with 65°C agarose) on the top of the non-denaturing 3% to 34% concave gradient polyacrylamide gels, followed by electrophoresis in the second dimension. Gels (3 mm thick) were prepared in a Hoefer gel-caster (SE-600). Electrophoresis was carried out in a Hoefer SE-600 electrophoresis apparatus (Amersham Pharmacia Biotech) for 24 hours at 250 V in buffer containing 90 mmol/L Tris, 80 mmol/L boric acid, and 2.5 mmol/L EDTA, pH 8.3. 125I-labeled molecular weight standard proteins (Pharmacia High Molecular Weight Standard) were applied in the middle of each polyacrylamide gel. Electrotrodeños onto nitrocellulose membranes (BA-S83, 0.2 μm, Schleicher & Schuell) was carried out at 30 V for 24 hours at 10°C. Posttransfer membrane operations included fixing with 0.3% glutaraldehyde in PBS for 10 minutes, rinsing with Tris-buffered PBS (PBST), blocking with 5% dry milk for 10 minutes, and incubating with nonspecific polyclonal goat anti-human apoA-I sera for 6
hours. Unbound antibody was removed by washing 3 times for 3 minutes each with PBST, followed by incubation with 125I-labeled rabbit anti-goat F(ab)2 IgG fragment overnight. Membranes then encircled HDL subpopulations. Data were expressed as pixels population was delineated. The program automatically measured the ratios of apoA-I to LDL-C (in milligrams apoA-I per deciliter plasma) were calculated by integrating the designated areas, each HDL subpopulation was further separated according to HDL-C level (in milligrams of HDL-C per deciliter plasma). The apoA-I concentrations were similar between the 2 groups. We calculated the ratios of apoA-I to α1 and of HDL-C to α1 and found that both ratios were significantly (P<0.001) higher in the CHD group (by 57% and 36%, respectively) than in the control group.

To test the hypothesis that HDL-C concentration has an influence on the values of the above-measured parameters, both groups were further separated according to HDL-C level into low (HDL-C ≤35 mg/dL) and normal (HDL-C >35 mg/dL) groups. There were 24 control (30.4%) and 37 CHD (48.7%) subjects in the low HDL-C group and 55 control (69.6%) and 39 CHD (51.3%) subjects in the normal HDL-C group. In the low HDL-C group, CHD subjects had significantly higher total cholesterol (20%, P<0.05) and significantly lower HDL-C (13%, P<0.05) concentrations compared with HDL-C-matched control subjects (Table 4). LDL-C, triglyceride, and apoA-I levels were higher by 13%, 15%, and 5%, respectively, but these differences were not significant. In the normal HDL-C group, only the average HDL-C concentration was significantly different (−9%, P<0.05) in CHD patients compared with control subjects. The ratios of LDL-C to HDL-C were higher in CHD patients in the low (21%) and normal (9%) HDL-C groups, but these differences were not significant (Table 4).

When the apoA-I–containing HDL subpopulations were compared between control and CHD subjects, very similar patterns were found in low and normal HDL-C groups (Table 5), and the differences resembled those obtained when all CHD patients were compared with all control individuals. In CHD patients with low HDL-C levels compared with control subjects, α1, pre-α1, pre-α2, and pre-α3 subpopulations were significantly lower (P<0.001), by −30%, −48%, −33%, and −32%, respectively, whereas pre-β1 and pre-β2 were significantly higher by 40% (P<0.05) and 21% (P<0.01), respectively (Table 5). Levels of pre-β2 (7%) and α2 (8%) were not significantly different between cases and controls with low HDL-C levels. In patients with normal HDL-C levels, the α1, pre-α1, pre-α2, and pre-α3 subpopulations were also significantly (P<0.001) lower by −29%, −44%, −29%, and −29%, respectively, whereas α1 was significantly higher by 21% (P<0.001), and the concentrations of pre-β1, pre-β2, and α2 were not significantly different when CHD cases were compared with the appropriate controls. The ratios of apoA-I to α1 and of HDL-C to α1 were significantly higher in CHD patients in the low and normal HDL-C groups by 57%, 37%,
TABLE 4. Comparison of Plasma Lipid Values After Subjects Were Stratified Into Low (≤35 mg/dL) and Normal (>35 mg/dL) HDL-C Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=24)</th>
<th>CHD (n=37)</th>
<th>Change, %</th>
<th>Control (n=55)</th>
<th>CHD (n=39)</th>
<th>Change, %</th>
<th>Change (Low/Normal), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>51.4±11.7</td>
<td>61.3±10.5</td>
<td>19†</td>
<td>54.1±13.0</td>
<td>62.1±8.9</td>
<td>15†</td>
<td>−5 −1</td>
</tr>
<tr>
<td>Plasma lipids, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>199.1±34.8</td>
<td>239.4±94.1</td>
<td>20‡</td>
<td>218.1±38.2</td>
<td>225.1±64.5</td>
<td>3</td>
<td>−8 6</td>
</tr>
<tr>
<td>LDL-C</td>
<td>135.9±27.6</td>
<td>153.6±70.8</td>
<td>13</td>
<td>149.1±35.3</td>
<td>151.7±51.8</td>
<td>2</td>
<td>−9 1</td>
</tr>
<tr>
<td>HDL-C</td>
<td>32.3±2.2</td>
<td>30.5±3.2</td>
<td>−6‡</td>
<td>48.4±10.6</td>
<td>43.9±6.4</td>
<td>−9‡</td>
<td>−33 −31</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>154.0±61.7</td>
<td>176.6±97.6</td>
<td>15</td>
<td>105.3±58.1</td>
<td>108.4±81.9</td>
<td>3</td>
<td>54 37</td>
</tr>
<tr>
<td>ApoA-I, mg/dL</td>
<td>103.3±11.5</td>
<td>108.5±12.5</td>
<td>5</td>
<td>127.9±19.1</td>
<td>128.3±15.0</td>
<td>&lt;1</td>
<td>−19 −15</td>
</tr>
<tr>
<td>HDL-C/HDL-C</td>
<td>4.2±0.8</td>
<td>5.1±2.4</td>
<td>21</td>
<td>3.2±0.9</td>
<td>3.5±1.2</td>
<td>9</td>
<td>31 46</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*Logarithmically transferred for analyses.
†P<0.01 and ‡P<0.05.

TABLE 5. Comparison of ApoA-I–Containing HDL Subpopulations After Subjects Were Stratified Into Low (<35 mg/dL) and Normal (>35 mg/dL) HDL-C Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=24)</th>
<th>CHD (n=37)</th>
<th>Change, %</th>
<th>Control (n=55)</th>
<th>CHD (n=39)</th>
<th>Change, %</th>
<th>Change (Low/Normal), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-β1, mg apoA-I/βVLDL plasma</td>
<td>5.5±3.0</td>
<td>7.7±3.8</td>
<td>40*</td>
<td>8.8±5.3</td>
<td>9.3±4.5</td>
<td>6</td>
<td>−37 −17</td>
</tr>
<tr>
<td>Pre-β2, mg apoA-I/βVLDL plasma</td>
<td>4.0±2.0</td>
<td>3.7±1.6</td>
<td>−7</td>
<td>3.7±1.7</td>
<td>4.3±1.9</td>
<td>16</td>
<td>8 −14</td>
</tr>
<tr>
<td>α1, mg apoA-I/βVLDL plasma</td>
<td>13.2±3.9</td>
<td>9.3±4.0</td>
<td>−30†</td>
<td>22.3±8.0</td>
<td>15.9±5.5</td>
<td>−29†</td>
<td>−41 −41</td>
</tr>
<tr>
<td>α2, mg apoA-I/βVLDL plasma</td>
<td>32.7±4.2</td>
<td>35.3±8.7</td>
<td>8</td>
<td>43.1±7.4</td>
<td>46.6±9.1</td>
<td>8</td>
<td>−24 −24</td>
</tr>
<tr>
<td>α3, mg apoA-I/βVLDL plasma</td>
<td>34.6±5.6</td>
<td>41.8±10.4</td>
<td>21‡</td>
<td>33.8±6.5</td>
<td>40.9±6.4</td>
<td>21†</td>
<td>2 2</td>
</tr>
<tr>
<td>Pre-α1, mg apoA-I/βVLDL plasma</td>
<td>3.3±1.8</td>
<td>1.7±1.0</td>
<td>−48‡</td>
<td>5.4±2.1</td>
<td>3.0±1.3</td>
<td>−44‡</td>
<td>−39 −43</td>
</tr>
<tr>
<td>Pre-α2, mg apoA-I/βVLDL plasma</td>
<td>5.4±2.1</td>
<td>3.6±1.2</td>
<td>−33‡</td>
<td>6.6±2.5</td>
<td>4.7±1.7</td>
<td>−29†</td>
<td>−18 −23</td>
</tr>
<tr>
<td>Pre-α3, mg apoA-I/βVLDL plasma</td>
<td>4.4±1.6</td>
<td>3.0±1.3</td>
<td>−32‡</td>
<td>4.1±1.7</td>
<td>2.9±1.2</td>
<td>−29†</td>
<td>7 3</td>
</tr>
<tr>
<td>ApoA-β/α1</td>
<td>8.3±1.8</td>
<td>13.0±5.1</td>
<td>57‡</td>
<td>6.4±2.2</td>
<td>9.3±4.2</td>
<td>44†</td>
<td>30 40</td>
</tr>
<tr>
<td>HDL-C/α1</td>
<td>2.7±0.8</td>
<td>3.7±1.4</td>
<td>37‡</td>
<td>2.4±0.9</td>
<td>3.2±1.4</td>
<td>33†</td>
<td>13 16</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*P<0.05, †P<0.001, and ‡P<0.01.

...and 44%, 33%, respectively, compared with ratios in the appropriate control groups.

The α1 and α2 HDL subpopulations were strongly correlated with HDL-C in control subjects (r=0.756 and r=0.714, respectively) and CHD subjects (r=0.774 and r=0.575, respectively). Concentration of the α1 subpopulation did not correlate with HDL-C. In addition, a very strong positive correlation was found between the α1 and α2 subpopulations and apoA-I levels in control subjects (r=0.832 and r=0.852, respectively) and CHD subjects (r=0.600 and r=0.798, respectively). The concentration of the α1 subpopulation was weakly correlated with apoA-I values in both groups. In control and CHD groups, the α1 subpopulation correlated negatively with the plasma triglyceride level (r=-0.476 and r=-0.517, respectively), whereas the α2 subpopulation correlated positively with the plasma triglyceride level (r=0.380 and r=0.230, respectively). No correlation was found between the α-mobility HDL subpopulations and either total cholesterol or LDL-C. In Figure 1, we present the regression lines and the 95% CIs around the regression lines for the various α-mobility subpopulations versus HDL-C and plasma total apoA-I for control subjects superimposed with the x-y scatterplot of the same parameters for CHD subjects. The majority of data points for CHD subjects of α1 versus HDL-C (66%) and apoA-I (86%) were below the 95% CIs calculated for control individuals. The majority of data points on CHD subjects with regard to α1 versus HDL-C (67%) and apoA-I (78%) were above the 95% CIs calculated for control individuals. Data points of cases on α2 subpopulations versus HDL-C (42%) and α1 versus apoA-I (38%) also showed a trend for values above the 95% CIs of control subjects.

Because earlier studies on the relation of HDL subspecies and CHD used ultracentrifugally separated subfractions, we compared the 2 methodologies. Ultracentrifugally separated HDL2 (density [d]<1.25) and HDL3 (d=1.25 to 1.24) were subjected to 2D nondenaturing gel electrophoresis (Figure 2). The majority of HDL2 was found in the α1 and pre-α1 positions, whereas HDL3 was a composite of the smaller α2, α3, pre-α2, pre-α3, and pre-β subpopulations. All of the pre-β2 and some apoA-I from the other particles were found...
in the d>1.21 g/mL fraction in accordance with our previous observation. Because it is known that β-blockers can affect lipoproteins, we examined HDL subtypes in CHD patients on and off β-blockers and saw no significant effects.

Discussion

The inverse association between the incidence of CHD and HDL has been known for decades. In the last 2 decades, many laboratories have tried to assess whether any of these subpopulations have a stronger relationship with risk for CHD than others. Despite conflicting data, as Silverman et al reviewed in 1993 and Montali et al summarized in 1994, many investigators believe that measuring HDL subfractions may provide additional information about atherosclerotic risk as well as about the underlying physiological mechanism responsible for that risk.

In the present study, we investigated the relationship between plasma lipid levels and the apoA-I–containing HDL subpopulations, separated by nondenaturing 2D gel electrophoresis, immunoblot, and image analysis as described in Methods.
Therefore, CHD subjects in the low HDL-C group were responsible for the significantly higher total cholesterol in the combined CHD group compared with the combined control group. Similar to total cholesterol, differences in the concentrations of LDL-C and triglycerides and the ratio of LDL-C to HDL-C were greater in low HDL-C cases than in cases with normal HDL-C levels compared with the appropriate control groups. Because the ratios of LDL-C to HDL are dependent on HDL-C concentrations, this ratio gives more precise information about risk for CHD when CHD patients are compared with HDL-C–matched control individuals. In contrast, HDL-C α1 ratio did not show this discriminative difference between groups with different HDL-C levels and was significantly lower in CHD cases in the low and normal HDL-C groups.

As we discussed earlier, the major HDL constituents, apoA-I and HDL-C, did not differ strikingly between control and CHD subjects. However, a major reorganization of the apoA-I– containing HDL subpopulations was observed in CHD patients compared with control individuals (Tables 3 and 5). The α1 and all of the pre-α subpopulations were significantly lower, whereas the α2 subpopulation was significantly higher in CHD subjects compared with control subjects. The differences in the concentrations of the α2 and the pre-β mobility subpopulations were not significant between the 2 groups. It has to be noted that there were significantly higher pre-β1 HDL subpopulations in CHD subjects than in control subjects when percent distribution was calculated. These data suggest that in CHD subjects the lipid-poor pre-β1 maturation into larger discoidal HDL particles (probably α1) is impaired. Because ~85% of apoA-I is in α mobility particles, our focus was on differences in these particles. Previously, we have reported that neither the α1 nor the pre-α–mobility HDL subpopulations contain apoA-II; therefore, they are LpA-I particles. LpA-I particles appear to play a pivotal role in reverse cholesterol transport and in the development of CHD. Our finding of decreased large LpA-I particles, including α1 and pre-α1 in CHD subjects versus control subjects supports recently published observations of Decossini et al.45

We have also demonstrated that subjects with low (<35 mg/dL) and normal (≥35 mg/dL) HDL-C levels have very different distributions of apoA-I– containing HDL subpopulations. Therefore, we examined whether the differences in the HDL subpopulation profiles between control and CHD subjects are only reflections of the differences in HDL-C levels. The subgroup analyses proved that the differences in HDL subpopulation profile between the control and CHD groups are still significant after the HDL-C level is taken into account. The α2 subpopulation is decreased by 41% in the low HDL-C groups compared with the normal HDL-C groups and is further decreased by about another 30% in CHD individuals compared with HDL-C–matched control subjects (Table 5).

Our results indicate that despite the decrease in apoA-I and HDL-C, the extensively decreased α2 (−35%) resulted in increased ratios of apoA-I to α1 (57%) and HDL-C to α1 (36%) in CHD individuals (Table 2). The differences in these ratios were still significant between controls and cases after subgrouping individuals into low and normal HDL-C groups (Table 5). The lower α1 and higher α1 concentrations in CHD subjects were also a characteristic for the group and independent of HDL-C level (Figure 1). These results indicate that some patients have CHD caused by factors that do not alter the lipid and HDL subpopulation profile. However, the majority of normolipidemic CHD patients have an altered distribution of HDL subpopulations.

On the basis of our results, we may be able to explain the discrepancies among laboratories on the possible role of HDL subclasses in the development of CHD. By our 2D analysis, the majority of HDL2 is in the α1 and pre-α1 positions (Figure 2). Earlier, we demonstrated that α1- and pre-α1 consist of LpA-I particles. Data generated by ultracentrifugation, immunoaffinity chromatography, or non-denaturing gel electrophoresis indicate lower levels of these particles in CHD individuals compared with control subjects.21–23 These findings are understandable, inasmuch as α1 is a homogeneous subpopulation and shows a very strong positive correlation with HDL-C and apoA-I concentrations, which are usually lower in CHD subjects. However, the picture is more complicated for HDL1, because it consists of many different HDL subspecies (mainly α2 and α3) of varying sizes and apolipoprotein composition (Figure 2). The concentration of these particles is differentially altered (α1 decreases, whereas α2 increases) in CHD individuals compared with control subjects.

Data generated in the present study on normolipidemic subjects support our hypothesis that the apoA-I– containing HDL subpopulation profile, obtained by 2D non-denaturing gel electrophoresis, is significantly different between control and CHD individuals. These differences were not significantly influenced by HDL-C or apoA-I concentrations. Our research findings support the concept that compared with control subjects, CHD patients not only have HDL deficiency but also have a major rearrangement in the HDL subpopulations with significantly lower α1 and pre-α1,1,3 (LpA-I) and significantly higher α1 (LpA-I/A-II) particles.

Acknowledgments

This research was supported by a grant from Parke Davis, Morris Plain, NJ, and by the National Institutes of Health/National Heart, Lung, and Blood Institute (HL-56160). The authors wish to thank Dana Bass for her excellent help in collecting medical histories on CHD patients.

References


Distribution of ApoA-I–Containing HDL Subpopulations in Patients With Coronary Heart Disease

Bela F. Asztalos, Paul S. Roheim, Richard L. Milani, Michael Lefevre, Judith R. McNamara, Katalin V. Horvath and Ernst J. Schaefer

doi: 10.1161/01.ATV.20.12.2670

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/20/12/2670

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/