Distribution of Subclasses of HDL Containing ApoA-I Without ApoA-II (LpA-I) in Normolipidemic Men and Women

Nicolas Duverger, Daniel Rader, H. Bryan Brewer, Jr

Abstract Women have significantly higher plasma concentrations of high-density lipoprotein (HDL) and apolipoprotein (apo) A-I than men. Human HDL consists of two major species of apoA-I-containing lipoproteins: LpA-I (lipoprotein containing apoA-I but not apoA-II) and LpA-I:A-II (lipoprotein containing both apoA-I and apoA-II). LpA-I is itself heterogeneous and contains several subclasses of different size and composition. We analyzed LpA-I subclasses in 12 male and 12 female healthy normolipidemic adults. LpA-I concentrations were significantly higher in women (72.4 ± 5.6 mg/dL) than in men (50.2 ± 2.2 mg/dL) (P < 0.05). LpA-I was primarily isolated from fasting plasma by immunoaffinity chromatography. Gel filtration chromatography was then used to isolate LpA-I subclasses based on size. Three major subclasses were eluted: large, medium, and small LpA-I. No differences between men and women in the size or composition of individual LpA-I subclasses were observed. In contrast, the distribution and plasma concentration of LpA-I subclasses were significantly different between men and women. As a fraction of total LpA-I, the large LpA-I was significantly higher (68.0% to 48.4%) and the medium LpA-I was significantly lower (26.4% to 44.9%) in women than in men. The fraction of small LpA-I was not significantly different. Plasma concentrations of large LpA-I in women (49.2 mg/dL) were twice that in men (24.3 mg/dL), whereas plasma concentrations of medium LpA-I (19.1 mg/dL versus 22.5 mg/dL) and small LpA-I (4.0 mg/dL versus 3.0 mg/dL) were similar in women and men. The activities and distribution of lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) were similar between women and men. The majority of plasma LCAT and CETP were associated with large LpA-I. In conclusion, the higher HDL cholesterol and apoA-I levels in normolipidemic women compared with men are primarily associated with elevation in a specific subclass, the large LpA-I particles. This suggests that large LpA-I may be a more specific marker for risk of premature coronary heart disease. (Arterioscler Thromb. 1994;14:1594-1599.)

Key Words • immunoaffinity chromatography • gel filtration chromatography • apolipoprotein A-I • cholesteryl ester transfer protein • lecithin:cholesterol acyltransferase

Plasma levels of high-density lipoprotein (HDL) cholesterol are inversely correlated with the risk of premature coronary heart disease (CHD).1,2 and it has been suggested that HDL is directly protective against the development of CHD. However, not all individuals with low levels of HDL appear to be at increased risk, leading to the concept that specific HDL subfractions may be better predictors for CHD risk.

The major apolipoproteins in HDL are apolipoprotein (apo) A-I and apoA-II. By using immunosorption techniques, HDL subclasses have been isolated according to their apolipoprotein content. HDL consists of two major classes of apoA-I-containing lipoproteins: lipoproteins that contain both apoA-I and apoA-II (LpA-I:A-II) and lipoproteins that contain apoA-I but no apoA-II (LpA-I).3,4 The plasma concentrations of HDL cholesterol and apoA-I are higher in women than in men,5,6 whereas plasma levels of apoA-II are similar. The sex differences in apoA-I levels are reported to be due to a difference in LpA-I and not in LpA-I:A-II.7,8 This difference may be related to the fact that CHD is much less common in premenopausal women than men.

Several studies have shown that LpA-I is itself heterogeneous, and we have characterized three major subclasses based on size.9 In the present study, we have compared the distribution, composition, and size of these LpA-I subclasses in normolipidemic men and women. We found that the higher levels of LpA-I in women are due solely to elevated concentrations of the largest LpA-I subclass and that individual particle composition and size do not differ between men and women.

Methods

Subjects

The study subjects were recruited through the National Institutes of Health normal volunteer program. They were college-aged (19 through 23 years old) students with no clinical symptoms who underwent a battery of screening blood tests to ensure that they had no subclinical disease. None of the study subjects were taking oral contraceptives, and none were smokers. Study subjects were placed on a controlled metabolic diet containing 47% carbohydrate, 37% fat, 16% protein, and 200 mg cholesterol per 1000 kcal for 14 days before the study. Fresh blood was collected from 12 men and 12 women after a 12-hour fast in tubes containing 0.1% EDTA. Plasma was obtained by centrifugation for 20 minutes at 2000 rpm at 4°C. All lipoprotein isolations were performed at 4°C.

Immunooaffinity Chromatography

Immunosorbents were prepared by coupling mixtures of monoclonal antibodies to apoA-I (A05-A17-A30) and apoA-II (G03-G05-G11) to CNBr-Sepharose 4B (Pharmacia). Lipo-
proteins were isolated in phosphate-buffered saline (PBS) containing 1 mmol/L EDTA and 0.1% sodium azide by an immunoaffinity chromatography procedure with the following modifications. Plasma aliquots were adsorbed sequentially with the anti-apoA-II immunosorbent to remove all LpA-I:A-II and then with the anti-apoA-I immunosorbent to retain all remaining LpA-I. Lipoproteins bound to immunosorbent were separately eluted with 3 mol/L NaSCN and immediately desalted with Sephadex G25 (Pharmacia). Three moles per liter NaSCN does not appear to affect the distribution of LpA-I subclasses, but no specific tests were performed in this study. All particles were dialyzed and concentrated in a Micro-Prodicon (Spectrum) against PBS and were filtered using a 0.22-μm Millipore filter. The lack of apoA-II in LpA-I was confirmed by enzyme-linked immunosorbent assay (ELISA).

**Gel Filtration Chromatography**

The individual LpA-I subclasses were isolated by gel filtration chromatography by using fast protein liquid chromatography with three Superose 12 columns and one Superose 6 column (Pharmacia) in series equilibrated in PBS at a flow rate of 9 ml/h. Samples containing 0.5 mg LpA-I were injected, and 0.2-ml fractions were collected. Protein was monitored using a UV monitor at 280 nm connected to the LCC-500 Plus. The system was calibrated for particle molecular weight and studies using human plasma and high-molecular-weight gel filtration standards (Pharmacia). The chromatographic system was calibrated every four runs. Each peak was detected and integrated by using a UV monitor at 280 nm. Liquid chromatography controller LCC-500 Plus (Pharmacia) was used for quantitative evaluation and reporting of chromatographic results. Using the distribution data and the apoA-I plasma concentration in LpA-I, we calculated the apoA-I plasma concentrations of the LpA-I subclasses. ApoA-I concentration correlated with OD% for all LpA-I subclasses. Total protein and apoA-I recoveries from the gel filtration were 95±6% and 96±5%, respectively.

**LCAT and CETP Activities**

Lecithin:cholesterol acyltransferase (LCAT) activities in plasma and isolated lipoproteins were measured by the apoA-I/lecithin/cholesterol proteoliposome substrate method with the modification that esterified and unesterified cholesterol were separated by thin-layer chromatography. Cholesterol ester transfer protein (CETP) activities in plasma and isolated lipoproteins were measured by the method of Albers et al. Briefly, samples were mixed with 0.1 mg of a-cholesteryl ester–HDL3 donor and 0.1 mg of low-density lipoprotein acceptor and incubated at 37°C in a shaking water bath for 5 hours. The reaction was stopped by chilling the tubes in ice. Donor and acceptor lipoproteins were separated by using the dextran sulfate/magnesium chloride precipitation procedure.

LCAT and CETP activity assays using exogenous substrates in excess reflect the mass of these two proteins. The LCAT and CETP activities in plasma and isolated lipoprotein fractions (LpA-I, LpA-I:A-II, and plasma without apoA-I– or apoA-II-containing lipoproteins) were normalized to plasma total protein concentration. Calculation of the distribution of LCAT and CETP activities between LpA-I and isolated fractions was performed. Total protein recovery was >90%. Recovery of LCAT activity (71±10%) and CETP activity (79±10%) in high-molecular-weight isolated particles was used for calculating distribution among these particles. Therefore, data concerning LpA-I subclasses were adjusted to allow for loss during the isolation procedures.

**Analytical Methods**

Total cholesterol, free cholesterol, triglyceride, and phospholipid levels were measured by using kits from Wako. The protein content of particles was measured according to the

<table>
<thead>
<tr>
<th>Table 1. Plasma Lipid and Apolipoprotein Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong> (n=12)</td>
</tr>
<tr>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Total cholesterol</td>
</tr>
<tr>
<td>Triglyceride</td>
</tr>
<tr>
<td>ApoB</td>
</tr>
<tr>
<td>HDL-C</td>
</tr>
<tr>
<td>ApoA-II</td>
</tr>
<tr>
<td>Total apoA-I</td>
</tr>
<tr>
<td>ApoA-I in LpA-I</td>
</tr>
<tr>
<td>ApoA-I in LpA-I:A-II</td>
</tr>
</tbody>
</table>

Apo indicates apolipoprotein; HDL-C, high-density lipoprotein cholesterol; LpA-I, lipoprotein containing apoA-I but not apoA-II; and LpA-I:A-II, lipoprotein containing both apoA-I and apoA-II. Values are mean±SEM and are expressed in milligrams per deciliter.

*P<0.05; †P<0.01.

The lipid and apolipoprotein levels are shown in Table 1. The levels of plasma total cholesterol, apoA-II, apoB, and apoA-I in LpA-I:A-II were similar in men and women. The plasma triglyceride levels were significantly higher in men than in women. The concentrations of apoA-I, HDL cholesterol, and apoA-I in LpA-I were significantly higher in women than in men. Composition and plasma concentration of isolated total LpA-I were shown in Table 2. Cholesterol, phospholipid, and protein composition in total LpA-I were significantly different between men and women. All plasma concentrations of total LpA-I components were significantly higher in women than in men.

**LpA-I Subclasses**

LpA-I subclasses from all 24 subjects were separated by gel filtration chromatography. Three subclasses were isolated from each subject: large LpA-I, medium LpA-I, and small LpA-I. Representative gel filtration profiles from one female and one male subject are shown in the Figure. Elution volumes of the LpA-I subclasses were similar in men and women, indicating that there were no sex differences with regard to particle sizes. By nondenaturing gradient polyacrylamide gel electrophoresis, using precast 4% to 30% gels (Pharmacia), Student's t test was used to evaluate the data.

**Results**

**Total LpA-I**

The lipid and apolipoprotein levels are shown in Table 1. The levels of plasma total cholesterol, apoA-II, apoB, and apoA-I in LpA-I:A-II were similar in men and women. The plasma triglyceride levels were significantly higher in men than in women. The concentrations of apoA-I, HDL cholesterol, and apoA-I in LpA-I were significantly higher in women than in men. Composition and plasma concentration of isolated total LpA-I were shown in Table 2. Cholesterol, phospholipid, and protein composition in total LpA-I were significantly different between men and women. All plasma concentrations of total LpA-I components were significantly higher in women than in men.
TABLE 2. Lipid and Protein Composition and Plasma Concentration of Total LpA-I

<table>
<thead>
<tr>
<th>Composition, Mass %</th>
<th>Plasma Concentration, mg/dL</th>
<th>Men</th>
<th>Women</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>19.9±0.8</td>
<td>23.3±0.8†</td>
<td>19.7±2.2</td>
<td>36.5±2.6†</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.9±0.5</td>
<td>7.5±0.6†</td>
<td>5.9±1.5</td>
<td>11.7±1.9†</td>
<td></td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>13.9±0.5</td>
<td>15.8±0.4†</td>
<td>13.8±1.8</td>
<td>24.7±2.2†</td>
<td></td>
</tr>
<tr>
<td>Phospholipid</td>
<td>22.1±0.6</td>
<td>23.4±0.3†</td>
<td>21.9±2.4</td>
<td>36.8±2.8†</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>4.9±0.2</td>
<td>5.1±0.2</td>
<td>4.8±2.5</td>
<td>8.1±2.5†</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>53.1±1.5</td>
<td>48.1±1.6†</td>
<td>52.7±6.2</td>
<td>75.5±5.3†</td>
<td></td>
</tr>
</tbody>
</table>

LpA-I indicates lipoprotein containing apolipoprotein (apo) A-I but not apoA-II. Values are mean±SEM.

*P<.05; †P<.01.

significant linear correlation between the HDL cholesterol level and the level of the large LpA-I subclass.

In contrast, the distribution of LpA-I subclasses was different between men and women (Figure and Table 4). As a fraction of total LpA-I, the large LpA-I was significantly higher (P<.001) and the medium LpA-I was significantly lower (P<.001) in women than in men. The fraction of small LpA-I was nonsignificantly lower in women. The plasma concentrations of the LpA-I subclasses were determined by multiplying the fractional distribution times the total plasma LpA-I concentration (Table 4). Women had higher levels of large LpA-I (P<.001), but there were no significant differences between men and women in concentrations of medium LpA-I or small LpA-I. The lipid and protein plasma concentrations of isolated LpA-I subclasses are shown in Table 5. In large LpA-I, all lipid and protein concentrations were significantly higher in women than men. In medium LpA-I and small LpA-I subclasses, no significant differences were observed.

LCAT and CETP

Mean LCAT activity in plasma was 94±5 nmol•h⁻¹·mL⁻¹ in men and 98±5 nmol•h⁻¹·mL⁻¹ in women. LCAT activity in total LpA-I and LpA-I subclasses is shown in Table 6. In plasma and total LpA-I, no significant differences were found between men and women. For both women and men, the CETP activity was found primarily in large LpA-I on a per milligram protein basis (P<.001). Using these data, we determined the distribution of CETP activity in plasma (Table 6). No significant differences were found between men and women.

### Discussion

Although the gender difference in incidence of CHD is well established, the reasons for this difference are less well understood. One possible factor is that women have higher HDL cholesterol levels than men.²⁻²¹ HDL exhibits considerable heterogeneity in size, density, and lipid and apolipoprotein composition,²² and some HDL subfractions have been proposed as being potentially more protective than others.²³ Therefore, it is of interest to establish whether differences in concentrations of specific HDL subfractions exist between men and women. The identification of differences more specific than HDL cholesterol might provide new insight into the basis of the link between HDL and CHD.

HDL can be separated by ultracentrifugation into several subfractions, such as HDL₂ and HDL₃.²² The plasma concentration of HDL₂ may be more strongly correlated with CHD risk than HDL₃.²⁴ However, other studies have not confirmed this association.²⁵ More recently, different HDL particles have been described based on apolipoprotein composition.

### Table 3. Lipid and Protein Composition of LpA-I Subclasses

<table>
<thead>
<tr>
<th>Composition</th>
<th>Large LpA-I</th>
<th>Medium LpA-I</th>
<th>Small LpA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>25.1±0.5</td>
<td>26.5±1.0</td>
<td>17.3±0.7</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>8.1±0.6</td>
<td>8.6±0.7</td>
<td>4.8±0.6</td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>17.0±0.6</td>
<td>17.9±0.8</td>
<td>12.5±0.8</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>24.0±0.7</td>
<td>24.7±0.9</td>
<td>19.1±0.6</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>5.4±0.3</td>
<td>5.3±0.4</td>
<td>5.5±0.3</td>
</tr>
<tr>
<td>Protein</td>
<td>45.5±2.2</td>
<td>43.4±2.5</td>
<td>58.1±3.0</td>
</tr>
</tbody>
</table>

LpA-I indicates lipoprotein containing apolipoprotein (apo) A-I but not apoA-II. Values are mean±SEM and are expressed as mass percent. See "Results" for definitions of LpA-I subclasses.
indicates that the two types of HDL particles (LpA-I and LpA-I:A-II) have different physiological properties and in vivo metabolism. ApoA-I is synthesized by both liver and intestine, but apoA-II is made only in the liver. The majority of plasma LCAT and CETP has been found in association with LpA-I, even though the plasma concentration is lower than that of LpA-I:A-II. ApoA-I:A-II appears to be a more effective substrate for hepatic lipase than LpA-I. Finally, apoA-I on LpA-I is catabolized at a faster rate than apoA-I on LpA-I:A-II, suggesting a greater “flux” of LpA-I particles.

LpA-I may be more directly protective against the development of atherosclerosis than LpA-I:A-II. Recent epidemiological study supports this concept. Moreover, overexpression of human apoA-I in transgenic mice protects against the development of atherosclerotic lesions, whereas overexpression of both human apoA-I and apoA-II does not prevent atherogenesis, despite similar plasma levels of HDL and apoA-I.

LpA-I concentrations are reported to be higher in women than men. In this study, we have confirmed this observation and further investigated the distribution and composition of the three major subclasses of LpA-I. LpA-I subclasses had similar particle sizes and composition in men and women. In contrast, the distribution of LpA-I subclasses was significantly different between men and women. LpA-I isolated from women consisted of 68% large LpA-I compared with only 48% of the LpA-I isolated from men. The plasma concentration of large LpA-I in women was twice that in men, whereas plasma concentrations of medium and small LpA-I were similar in men and women. Therefore, the increased levels of apoA-I and LpA-I in women are due solely to increased concentrations of the large LpA-I particle.

Several possible factors may be responsible for the higher levels of large LpA-I in women. The activities of lipoprotein lipase (LPL) and LCAT promote the formation of larger HDL, while the activities of hepatic lipase (HL) and CETP result in the formation of smaller HDL. To investigate the gender difference in large LpA-I concentrations, we measured the LCAT and CETP activities associated with the particles. We found no significant differences in the total plasma LCAT and CETP activities between women and men, consistent with reports of LCAT mass and CETP mass in both sexes. Furthermore, there were no differences between men and women in LCAT and CETP activities in the individual LpA-I subclasses. Hence, the difference in large LpA-I levels between women and men is unlikely to be due to differences in LCAT and CETP. Recently, McCall et al have proposed that a factor different from LCAT is secreted by HepG2 cells that modifies nascent HDL into predominantly HDL2b. As large LpA-I represents the majority of HDL2b, this factor could theoretically be responsible for the sex differences in large LpA-I concentrations. Sex differences and the effects of gonadal steroids on their expression in the activities of heparin-releasable lipases have been described. Postheparin LPL activity may be slightly higher in women than men, although LPL regulation is not dependent on steroid hormones. In contrast, postheparin HL activity may be significantly lower in women than men, a difference that appears to be due to differences in gonadal hormones. Therefore, lower HL activity in women might be responsible for the increased levels of large LpA-I. LpA-I:A-II is a preferred substrate for HL over LpA-I. We speculate that large LpA-I, which contains apoE, could also be a good substrate for HL.

### Table 4. Distribution and Plasma Concentrations of LpA-I Subclasses

<table>
<thead>
<tr>
<th>Subclass</th>
<th>Men</th>
<th>Women</th>
<th>Men</th>
<th>Women</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large LpA-I</td>
<td>48.4±6.3</td>
<td>68.0±6.8*</td>
<td>44.9±5.4</td>
<td>26.4±5.2*</td>
<td>6.6±1.7</td>
<td>5.6±2.6</td>
</tr>
<tr>
<td>Medium LpA-I</td>
<td>24±5</td>
<td>50±6*</td>
<td>23±4</td>
<td>19±4</td>
<td>3±1</td>
<td>4±2</td>
</tr>
</tbody>
</table>

**LpA-I indicates lipoprotein containing apolipoprotein (apo) A-I but not apoA-II. Values are mean±SEM and are expressed in milligrams per deciliter. See "Results" for definitions of LpA-I subclasses.**

### Table 5. Lipid and Protein Plasma Concentrations of LpA-I Subclasses

<table>
<thead>
<tr>
<th>Subclass</th>
<th>Men</th>
<th>Women</th>
<th>Men</th>
<th>Women</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large LpA-I</td>
<td>14.2±1.3</td>
<td>31.6±3.0*</td>
<td>7.0±0.9</td>
<td>6.5±1.4</td>
<td>0.22±0.02</td>
<td>0.25±0.03</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.6±0.8</td>
<td>10.3±1.7*</td>
<td>1.9±0.8</td>
<td>1.8±0.7</td>
<td>0.13±0.03</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>9.6±2.2</td>
<td>21.3±2.3*</td>
<td>5.1±0.8</td>
<td>4.6±1.0</td>
<td>0.10±0.02</td>
<td>0.11±0.03</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>13.6±2.0</td>
<td>29.3±2.9*</td>
<td>7.7±1.2</td>
<td>7.3±1.4</td>
<td>1.15±0.10</td>
<td>1.24±0.12</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>3.1±0.6</td>
<td>6.3±0.4*</td>
<td>2.2±0.6</td>
<td>2.0±0.5</td>
<td>0.05±0.02</td>
<td>0.05±0.02</td>
</tr>
<tr>
<td>Protein</td>
<td>25.7±5.2</td>
<td>51.7±8.5*</td>
<td>23.6±5.3</td>
<td>20.1±8.3</td>
<td>5.14±1.03</td>
<td>5.46±1.09</td>
</tr>
</tbody>
</table>

**LpA-I indicates lipoprotein containing apolipoprotein (apo) A-I but not apoA-II. Values are mean±SEM and are expressed in milligrams per deciliter. See "Results" for definitions of LpA-I subclasses.**

*P<.005.
This is consistent with the observation that LpA-I isolated from women contains more apoE than LpA-I from men7,8 and with our data that apoE is associated only with large LpA-I among the LpA-I subclasses.9

In conclusion, this study confirms that plasma concentrations of LpA-I are higher in women than men and further establishes that this difference is primarily due to higher concentrations of only the largest LpA-I subclass (large LpA-I). These data suggest that the large LpA-I concentration may be a better indicator of CHD risk than HDL cholesterol or total LpA-I concentration.

References


Distribution of subclasses of HDL containing apoA-I without apoA-II (LpA-I) in normolipidemic men and women.

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