Age- and Sex-related Differences in Lipoproteins Containing Apoprotein A-I

Takao Ohta, Shinzabro Hattori, Masaji Murakami, Soroku Nishiyama, and Ichiro Matsuda

We investigated age- and sex-related differences in the structure and composition of two species of lipoproteins that contain apoprotein (apo) A-I (A-I Lp): lipoproteins containing apo A-I and apo A-II (Lp A-I/A-II), and lipoprotein containing apo A-I but no apo A-II (Lp A-I), which were isolated by immunoaffinity chromatography. Sixty normolipidemic volunteers were assigned to one of three groups based on their ages and sexual maturation (Group A, prepubertal; Group B, puberty; and Group C, postpubertal). In A-I Lp, the levels of total cholesterol, cholesteryl ester, phospholipid, and apo A-I were lower in males during puberty and then remained stable. In Lp A-I/A-II, there were no age- or sex-related changes in lipids or apo A-II. Levels of apo A-I in the females were lower with advance in age, although significant differences were observed only between pre- and postpubertal subjects. In Lp A-I, the levels of total cholesterol, cholesteryl ester, phospholipid, and apo A-I were lower in males during puberty and remained stable thereafter, as in the case of A-I Lp. Therefore, the age- and sex-related differences observed in A-I Lp appear to be primarily due to the differences in Lp A-I/A-II. When we take into account the constancy of Lp A-I/A-II levels in all groups, the physiological function of A-I Lp (high density lipoprotein) in each individual may be limited by the Lp A-I levels.

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Apolipoprotein (apo) A-I and apo A-II are major apolipoprotein components of high density lipoproteins (HDL). The majority of these apolipoproteins in normolipidemic fasting plasma are present in the HDL fraction (density range 1.063 to 1.21 g/ml), although a minor portion is associated with apo B-containing lipoprotein and is present in the less dense fractions. Recent studies concerning HDL metabolism have shown the importance of apo A-I and apo A-II of HDL particles in determining physiological role and metabolic fate. Therefore, it seems that lipoproteins should be isolated by apolipoprotein composition and not by density.

In recent years, McVicar et al. and Cheung and Albers independently isolated apo A-I containing lipoproteins (A-I Lp) by immunoaffinity chromatography and found that A-I Lp had a chemical nature similar to that of HDL. Furthermore, Cheung and Albers divided A-I Lp into two subspecies using anti-apo A-I and anti-apo A-II immunoabsorbent columns: lipoproteins containing apo A-I and apo A-II (Lp A-I/A-II), and lipoproteins containing apo A-I but no apo A-II (Lp A-I). These lipoproteins also showed HDL-like characteristics in terms of particle size, electrophoretic mobility, and lipid and apolipoprotein composition, and they appeared to be more native than HDL because ultracentrifugation was not involved in the isolation. Thus, characterization of A-I Lp, Lp A-I/A-II, and Lp A-I should aid in elucidating the pathophysiological role of native HDL in atherosclerosis.

In a previous report, we described sex differences of lipid and apolipoprotein compositions of A-I Lp, Lp A-I/A-II, and Lp A-I in normolipidemic adults; lipid levels of A-I Lp and lipid and apo A-I levels of Lp A-I were higher in women than in men. This difference may be associated with the sex difference in the incidence of coronary artery disease, which is higher in men than in women before menopause. Therefore, the present study was designed to gain further insight into sex differences by studying prepubertal and pubertal children as well as mature adults. We found that while decreases in plasma A-I Lp and Lp A-I do occur at puberty in boys, no sex- or age-related differences occur in the case of Lp A-I/A-II.

Methods

Subjects

The 60 normolipidemic subjects studied were volunteers from the staff at Kumamoto University or their children. All were from different nonrelated families (nonconsanguineous). These subjects were divided into three groups based on age and degree of sexual maturation. Sexual maturation was determined by visual assessment of secondary sex characteristics, according to the method of Tanner. The rating for sexual maturation ranged from I to V (complete development) according to the stages of female breast or male genitalia development. Each group included ten males and ten females: Group A,
Tanner I and II, ages 6 to 10 years old; Group B, Tanner III and IV, ages 11 to 15 years old; Group C, Tanner V, ages 16 to 35 years old.

Diabetes mellitus, liver, and thyroid dysfunctions were absent in all subjects. There was no obesity (more than 20% of desirable weight). No subject was taking any drug known to influence lipoprotein metabolism, and there were no dietary restrictions. Informed consent was obtained from all subjects and both parents.

Venous blood was drawn into Vacutainer tubes containing disodium EDTA (1.5 mg/ml) after an overnight fast, and the plasma was promptly separated by low-speed centrifugation at 4°C.

Procedures

Isolation of Two Species of Apo A-I Containing Lipoprotein by Immunosorbent Column

The two species of apo A-I containing lipoproteins were isolated from plasma by combination of anti-apo A-I and anti-apo A-II immunosorbent columns described in detail previously. Briefly, fresh plasma (3 ml) was applied on an anti-apo A-I immunosorbent column (1.5 x 10 cm). After washing extensively with 0.01 M Tris, 0.5 M NaCl, 1 mM EDTA, pH 7.4 (buffer A), the column was eluted with 15 ml of 0.1 M acetic acid, 1 mM EDTA (pH 3.0) at a flow rate of 20 ml/h. Each effluent was immediately adjusted to pH 7.4 with 1.0 M Tris solution and dialyzed against 0.15 M NaCl and 1 mM EDTA, pH 7.4 (buffer B). Finally, the sample was concentrated to 9 ml in an Amicon ultrafiltration cell equipped with a PM-10 membrane. A portion (3 ml) of this sample was used for analysis of apolipoproteins and lipids. The remaining portion (6 ml) was used to separate the A-I Lp into Lp A-I-A-II and Lp A-I. The sample was applied on an anti-apo A-II immunosorbent column. The column was washed with buffer A to obtain the unbound fraction (Lp A-I). The bound fraction (Lp A-I-A-II) was eluted with 0.1 M acetic acid, 1 mM EDTA (pH 3.0). Both the unbound and bound fractions were dialyzed and then concentrated with an ultrafiltration apparatus (Amicon) to 6 ml in buffer B.

Protein and Lipid Analysis

The apo A-I, apo A-II, apo C-II, apo C-III, and apo E concentrations of plasma, A-I Lp, Lp A-I-A-II, and Lp A-I were measured by radial immunodiffusion assay as described previously. Cholesterol and triglyceride concentrations of plasma, A-I Lp, Lp A-I-A-II, and Lp A-I were analyzed on an ABA 100 Autoanalyzer (Abbott Laboratories) by enzymatic methods. Cholesteryl ester was measured by the enzymatic method and fluorometry. Phospholipid was analyzed by the method of Bartlett. The protein content of each fraction from the immunosorbent columns was determined by the method of Lowry et al.

Electrophoretic Analysis

Agarose gel electrophoresis was performed using a Pol-E Film system for lipoprotein electrophoresis (Coming) at pH 8.6, followed by staining with fat red 7B. Slab gel electrophoresis of lipoproteins isolated in 15% polyacrylamide gels containing 0.1% sodium dodecyl sulfate was performed according to the method of Weber and Osbom. The Stokes diameters of the isolated particles were estimated by gradient polyacrylamide gel electrophoresis on Pharmacia precast PAA 4/30 gels according to the procedure of the manufacturer (Pharmacia, Uppsala, Sweden). Thyroglobulin, apoferritin, catalase, lactate dehydrogenase, and bovine albumin (Pharmacia) were used as the calibrating proteins. The Stokes diameters of these calibrating proteins are: thyroglobulin, 17.0 nm; apoferritin, 12.2 nm; catalase, 10.4 nm; lactate dehydrogenase, 8.2 nm; and bovine albumin, 7.5 nm.

Statistical Evaluation

The Wilcoxon signed rank test was used to evaluate the data.

Results

Plasma Lipid and Apolipoprotein Levels

The plasma lipid and apolipoprotein levels in the subjects are summarized in Table 1. In Group B, the levels of total cholesterol and phospholipid in males were significantly lower than those in females (p<0.01 or 0.005). In Group C, triglyceride levels in males were significantly higher than those in females (p<0.05).

Concerning the relationship among the three groups, the levels of total cholesterol in Group C males were significantly higher than those in Groups A and B males (p<0.025 or 0.005). Phospholipid levels in Group B males were significantly lower than those in Groups A and C males, and those in Group A females were significantly lower than those in Groups B and C females (p<0.05). Apo A-I levels in Group A males were significantly higher than those in Groups B and C males (p<0.05 or 0.025). The groups did not differ significantly in plasma levels of apo C-II, apo C-III, or apo E.

All of the plasma lipid and apolipoprotein levels in the subjects were within normal range for Japanese.

Lipid and Apolipoprotein Compositions of Lipoproteins Containing Apo A-I

The lipid and apolipoprotein compositions of A-I Lp are shown in Table 2. In Groups B and C, the levels of total cholesterol, cholesteryl ester, and phospholipid were significantly lower in males than in females (p<0.01 or 0.005). The levels of total cholesterol, cholesteryl ester, and phospholipid in Group A males were significantly higher than those in Groups B and C males (p<0.05 or 0.01). In Group C, apo E levels were lower in males than in females (mean±SEM: males, 1.15±0.13; females, 1.83±0.24 mg/dl; p<0.05). The levels of other apolipoproteins were similar in males and females in all groups, and there were no differences among groups. The molar ratio of apo A-I to apo A-II was slightly higher in females in both Groups B and C, but the differences were only significant in Group B (mean±SEM: males, 2.5±0.08; females, 2.78±0.08; p<0.01).

Lipid and Apolipoprotein Compositions of Lipoproteins Containing Apo A-I and Apo A-II

The lipid and apolipoprotein compositions of Lp A-I-A-II are shown in Table 3. The levels of all lipids were similar...
Table 1. Plasma Lipids and Apolipoprotein Concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Mean age* (yrs)</th>
<th>Total cholesterol</th>
<th>Triglyceride</th>
<th>Phospholipid</th>
<th>Apo A-l</th>
<th>Apo A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>8.4</td>
<td>159.3±5.0</td>
<td>82.5±12.5</td>
<td>190.7±5.4</td>
<td>148.9±4.6</td>
<td>34.3±1.6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>8.2</td>
<td>160.3±5.8</td>
<td>74.9±9.5</td>
<td>184.1±3.8</td>
<td>141.1±6.4</td>
<td>34.0±1.0</td>
</tr>
<tr>
<td>B</td>
<td>M</td>
<td>13.0</td>
<td>150.4±5.9</td>
<td>73.5±6.0</td>
<td>170.1±5.6</td>
<td>132.6±4.6</td>
<td>32.8±1.5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12.7</td>
<td>174.8±3.5</td>
<td>80.8±13.0</td>
<td>196.3±4.0</td>
<td>139.7±4.2</td>
<td>31.2±1.3</td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>24.4</td>
<td>185.0±8.2</td>
<td>83.5±11.0</td>
<td>189.9±7.1</td>
<td>136.6±3.6</td>
<td>33.3±1.2</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>23.8</td>
<td>178.1±9.3</td>
<td>54.3±5.0</td>
<td>198.4±7.5</td>
<td>134.6±4.5</td>
<td>30.0±1.8</td>
</tr>
</tbody>
</table>

Values are mg/dl (mean±SEM).
There were 10 males and 10 females in each group.
*The age range is in parentheses, a, p<0.05; b, p<0.025; c, p<0.01; d, p<0.005.

Table 2. Lipid and Apolipoprotein Concentrations of Lipoproteins Containing Apolipoprotein A-I

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Total cholesterol</th>
<th>Cholesteryl ester</th>
<th>Triglyceride</th>
<th>Phospholipid</th>
<th>Apo A-l</th>
<th>Apo A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>71.9±2.3</td>
<td>52.5±1.5</td>
<td>19.5±2.5</td>
<td>120.9±4.6</td>
<td>147.3±4.6</td>
<td>33.6±1.6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>68.1±4.8</td>
<td>49.4±3.7</td>
<td>18.4±2.1</td>
<td>109.1±6.0</td>
<td>142.2±6.4</td>
<td>33.2±1.0</td>
</tr>
<tr>
<td>B</td>
<td>M</td>
<td>62.9±1.6</td>
<td>46.4±1.1</td>
<td>16.1±1.7</td>
<td>100.2±3.5</td>
<td>131.7±4.6</td>
<td>31.8±1.5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>74.8±2.9</td>
<td>55.7±1.9</td>
<td>18.1±2.3</td>
<td>121.5±3.6</td>
<td>138.5±4.2</td>
<td>30.7±1.3</td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>62.7±3.0</td>
<td>46.6±2.0</td>
<td>18.0±3.4</td>
<td>97.1±4.8</td>
<td>135.1±3.0</td>
<td>33.1±1.1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>76.3±3.1</td>
<td>55.9±0.9</td>
<td>14.8±1.3</td>
<td>122.2±3.5</td>
<td>133.5±4.1</td>
<td>29.6±1.9</td>
</tr>
</tbody>
</table>

Values are mg/dl (mean±SEM).
Each group comprised 10 female and 10 male subjects.
a, p<0.05; b, p<0.01; c, p<0.005.

Table 3. Lipid and Apolipoprotein Concentrations of Lipoproteins Containing Both Apolipoprotein A-I and Apoprotein A-II

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Total cholesterol</th>
<th>Cholesteryl ester</th>
<th>Triglyceride</th>
<th>Phospholipid</th>
<th>Apo A-I</th>
<th>Apo A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>40.4±1.1</td>
<td>30.6±0.9</td>
<td>9.4±1.1</td>
<td>69.6±1.6</td>
<td>86.5±2.5</td>
<td>34.0±1.6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>38.8±1.7</td>
<td>29.9±1.9</td>
<td>9.8±1.2</td>
<td>66.0±2.7</td>
<td>85.8±3.3</td>
<td>33.5±1.0</td>
</tr>
<tr>
<td>B</td>
<td>M</td>
<td>39.2±1.3</td>
<td>29.8±0.9</td>
<td>8.9±0.9</td>
<td>65.8±2.8</td>
<td>84.8±3.0</td>
<td>31.7±1.5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>39.8±0.9</td>
<td>30.8±0.8</td>
<td>9.2±1.5</td>
<td>67.8±1.4</td>
<td>80.4±3.2</td>
<td>30.9±1.3</td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>38.7±1.2</td>
<td>29.4±0.9</td>
<td>9.3±1.7</td>
<td>63.0±1.9</td>
<td>84.2±3.0</td>
<td>32.5±1.1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>40.4±1.3</td>
<td>30.5±1.0</td>
<td>7.5±0.8</td>
<td>68.9±3.7</td>
<td>75.0±2.9</td>
<td>29.8±1.2</td>
</tr>
</tbody>
</table>

Values are mg/dl (mean±SEM).
Each group comprised 10 female and 10 male subjects.
a, p<0.05; b, p<0.01.

in both sexes in all groups, and differences among the groups were nil. In Group C, the levels of apo A-I were higher in males than in females, but apo E levels were lower in males than in females (apo E mean±SEM: males, 0.76±0.06; females, 1.37±0.21 mg/dl; p<0.05).
Apo A-I levels in Group C females were significantly lower than those in Group A females (p<0.01). Apo C-III levels in Group B females were significantly higher than those in Groups A and C (mean±SEM: Group B, 2.82±0.14; Group A, 2.33±0.17; Group C, 2.22±0.20 mg/dl; p<0.05).
The levels of apo A-II and apo C-II were similar in both sexes, in all groups, and among groups. The molar ratio of apo A-I to apo A-II was fairly constant in all groups.

Lipid and Apolipoprotein Compositions of Lipoproteins Containing Apo A-I Only

The lipid and apolipoprotein compositions of Lp A-I are shown in Table 4. In Groups B and C, the levels of total cholesterol, cholesteryl ester, and phospholipid were significantly lower in males than in females (p<0.05 or p<0.005). The triglyceride levels were similar in males and females in all groups, and there was no difference among groups. The
Table 4. Lipid and Apolipoprotein Concentrations of Lipoproteins Containing Only Apolipoprotein A-I

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Total cholesterol</th>
<th>Cholesteryl ester</th>
<th>Triglyceride</th>
<th>Phospholipid</th>
<th>Apo A-I</th>
<th>Apo A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>31.4±2.3</td>
<td>21.9±1.5</td>
<td>10.2±1.6</td>
<td>51.3±3.7</td>
<td>60.4±3.4</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>29.3±3.9</td>
<td>19.4±2.5</td>
<td>8.8±1.0</td>
<td>44.2±4.9</td>
<td>55.2±4.0</td>
<td>0±0</td>
</tr>
<tr>
<td>B</td>
<td>M</td>
<td>23.7±1.2</td>
<td>16.7±0.9</td>
<td>7.2±0.9</td>
<td>34.4±2.5</td>
<td>47.7±2.0</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>34.9±2.3</td>
<td>25.0±1.6</td>
<td>9.0±0.8</td>
<td>53.6±3.4</td>
<td>59.3±2.3</td>
<td>0±0</td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>24.0±1.9</td>
<td>17.2±1.3</td>
<td>8.7±1.8</td>
<td>34.1±3.4</td>
<td>50.7±2.1</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>35.9±1.4</td>
<td>25.3±0.9</td>
<td>7.1±0.8</td>
<td>55.9±2.3</td>
<td>59.6±3.0</td>
<td>0±0</td>
</tr>
</tbody>
</table>

Values are mg/dl (mean±SEM). Each group comprised 10 female and 10 male subjects.

Table 5. Lipid Composition of Lipoproteins Containing Apolipoproteins A-I and A-II and of Lipoproteins Containing Apolipoprotein A-I Only

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>% of total lipid</th>
<th>Lipid ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total cholesterol</td>
<td>Cholesteryl ester/total cholesterol</td>
</tr>
<tr>
<td>A</td>
<td>M</td>
<td>34.8±0.7</td>
<td>75.6±0.7</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>35.6±0.8</td>
<td>70.2±1.2</td>
</tr>
<tr>
<td>B</td>
<td>M</td>
<td>34.4±0.6</td>
<td>75.8±1.4</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>37.4±0.9</td>
<td>71.7±1.0</td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>34.8±0.5</td>
<td>76.1±0.6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>36.3±0.9</td>
<td>72.1±0.9</td>
</tr>
</tbody>
</table>

Values are mg/dl (mean±SEM). Each group comprised 10 female and 10 male subjects.

levels of total cholesterol, cholesteryl ester, and phospholipid in Group A males were significantly higher than those in Groups B and C males (p<0.05 or 0.005).

Apo A-I levels in Groups B and C were significantly lower in males than in females (p<0.005), and apo A-I levels in Group A males were significantly higher than those in Groups B and C males (p<0.005). Apo C-III levels in Group B were significantly lower in males than in females (mean±SEM: males, 1.32±0.07; females, 2.12±0.24 mg/dl; p<0.05). Apo C-III levels in Group A males were significantly higher and levels in females were significantly lower than those in Group B counterparts (mean±SEM: Group A males, 1.60±0.12; females, 1.49±0.15; Group B males, 1.32±0.07; females, 2.12±0.24 mg/dl; p<0.05). The levels of apo C-II and apo E were similar in each group and among the groups.

Percent Lipid Compositions of Lipoproteins Containing Apolipoproteins A-I and A-II and of Lipoproteins Containing Apolipoprotein A-I Only

As shown in Table 5, the percent of phospholipid was significantly higher in Lp A-I/A-II than in Lp A-I except in the Group C females (p<0.005). In this group, the percent of phospholipid in Lp A-I was significantly lower in males than in females (p<0.005). The percent of triglyceride in all group males and Group A females was significantly lower in Lp A-I/A-II than in Lp A-I (p<0.05 or 0.005). In Group C, the percent of triglyceride in Lp A-I was significantly higher in males than in females (p<0.05). The percent of total cholesterol in Groups B and C males was significantly lower in Lp A-I/A-II than in Lp A-I (p<0.025). The ratios of cholesteryl ester to total cholesterol of Lp A-I in the three groups were significantly lower than those in Lp A-I/A-II (p<0.005) and there was no difference between sexes.

Apolipoprotein C-II/C-III Ratios in Both Types of Lipoproteins

As shown in Table 6, the apo C-II/apo C-III ratio in Lp A-I/A-II was significantly lower than in the case of Lp A-I (p<0.005).

Particle Diameter of Lipoproteins Containing Apolipoproteins A-I and A-II and of Lipoproteins Containing Apolipoprotein A-I Only

The Lp A-I had two distinct particle sizes: Stokes diameters of 11.1±0.4 nm and 8.8±0.2 nm (mean±SD). Lp A-I/A-II exhibited three distinct particle sizes: Stokes
we speculate that our conclusions may apply to the striking increase in HDL components. 22 Our female sub-
children, plasma testosterone in males and estradiol in levels. The relationships between sex hormones and A-1
vations on Japanese children. 21 Similar to Caucasian
ments, despite possible increases in plasma estradiol
and testosterone may be a significant determinant of plasma
analysis, we did compare our results with hormonal obser-
mechanism for the decrease of A-1 Lp in males during
stant suggest that phospholipid-rich LDL particles
increased during and after puberty in females. While the
because of different procedures used for isolation, all these data suggest a close
relationship between HDL2 and Lp A-I and between HDL3 and Lp A-I/A-II. Thus, the present findings that
major components of Lp A-I were higher in females than males during and after puberty and that major compo-
ents of Lp A-I/A-II (except apo A-I) were fairly constant suggest that phospholipid-rich LDL particles
increased during and after puberty in females. While the mechanism for the decrease of A-I Lp in males during puberty is not well understood, it has been proposed that plasma testosterone or the interaction of estradiol and testosterone may be a significant determinant of plasma
HDL-C levels. 20 Although we did not do a hormonal analysis, we did compare our results with hormonal observ-
ations on Japanese children. 21 Similar to Caucasian
children, plasma testosterone in males and estradiol in females begin to increase around 11 years of age. Thus, we speculate that our conclusions may apply to the components of A-I Lp. It has been reported that admin-
istering estrogen to premenopausal women results in a striking increase in HDL components. 22 Our female sub-
jects showed no age-related changes in A-I Lp compo-
ents, despite possible increases in plasma estradiol
levels. The relationships between sex hormones and A-I
Lp components in females seem to be more complex.

### Discussion

There are other reports on A-I Lp and its subspecies, which were isolated by immunoaffinity chromatography. 1,6 However, attention was directed to the chemical structures of these lipoproteins. Age- and sex-related differences and physiological functions were not documented. By contrast, our present study sheds light on these factors and provides new findings that sex differences of A-I Lp and Lp A-I were evident during the pubertal stage, as there was a decline in the major components of these lipoproteins in the male subjects.

In the literature, age- and sex-related changes were observed in plasma cholesterol, low density lipoprotein cholesterol (LDL-C), and HDL cholesterol (HDL-C); there was a significant decrease in HDL-C in males during puberty and a later increase in LDL-C. These changes led to decreased plasma cholesterol levels during puberty and increased levels after puberty in males. 1,6,21 In consideration of the similarity between HDL and A-I Lp, our lipid and apolipoprotein data of A-I Lp in males confirmed these reports and further extended the findings to phospholipid, cholesterol, ester, and apo A-I, although the apo A-I levels remained stable after puberty. In female subjects, the only age-related changes were in plasma phospholipid levels. This observation and the finding that phospholipid levels in females of A-I Lp were fairly constant suggest that phospholipid-rich LDL particles increased during and after puberty in females. While the mechanism for the decrease of A-I Lp in males during puberty is not well understood, it has been proposed that plasma testosterone or the interaction of estradiol and testosterone may be a significant determinant of plasma
HDL-C levels. 20 Although we did not do a hormonal analysis, we did compare our results with hormonal observ-
ations on Japanese children. 21 Similar to Caucasian
children, plasma testosterone in males and estradiol in females begin to increase around 11 years of age. Thus, we speculate that our conclusions may apply to the components of A-I Lp. It has been reported that admin-
istering estrogen to premenopausal women results in a striking increase in HDL components. 22 Our female sub-
jects showed no age-related changes in A-I Lp compo-
ents, despite possible increases in plasma estradiol
levels. The relationships between sex hormones and A-I
Lp components in females seem to be more complex.

Except for apo A-I, only apo E levels in A-I Lp after puberty were higher in females. Williams et al. 23 show that phospholipid liposomes acquire apo E in plasma and block cholesterol loading of cultured macrophages. Our results showing apo E in A-I Lp may be explained by the fact that phospholipid is rich in A-I Lp in females.

HDL2 (1.063<d<1.125), and HDL3 (1.125<d<1.21) are widely accepted as subspecies of HDL. HDL2 chole-
sterol is apparently higher in females than in males older than 20 years of age. 24,25 In addition, the levels of HDL2 cholesterol are strongly and inversely correlated with the incidence of coronary heart disease. 24,25,26 However, even in these lipoproteins, age- and sex-related changes in other components such as apolipoproteins, phospholipid, cholesterol ester, and triglyceride have remained obscure. Nestruck et al. 27 reported that lipoproteins containing apo A-I but no apo A-II (their fractions 1 and 2) were HDL2-like, and that lipoproteins containing apo A-I and apo A-II (their fractions 4 to 6) were HDL3-like, as determined from their hydrated densities. 27 Atmeh et al. 28 using immunoprecipitation procedures, found that approximately 70% of the particles in HDL2 contained apo A-I but no apo A-II, and that 67% and 33% of these particles were found in HDL2 and HDL3, respectively. They also found that HDL3 comprised mainly lipoproteins containing apo A-I and apo A-II. Although our Lp A-I and Lp A-I/A-II were not similar to their lipoproteins because of different procedures used for isolation, all these data suggest a close relationship between HDL2 and Lp A-I and between HDL3 and Lp A-I/A-II. Thus, the present findings that major components of Lp A-I were higher in females than males during and after puberty and that major compo-
ents of Lp A-I/A-II (except apo A-I) were fairly constant indicate that the characteristics of HDL2 and HDL3 reported 24,25,26 may depend mostly on those of Lp A-I and Lp A-I/A-II, respectively.

Huff et al. 29 found that apo C-II and apo C-III in HDL had a similar metabolic pathway, but the apo C in the HDL subspecies was not reported. Our present study shows that apo C-II and apo C-III were distributed unevenly in subspecies of A-I Lp, and the apo C-II/apo C-III ratio was higher in Lp A-I than in Lp A-I/A-II. These results suggest that, as in the case of whole HDL, apo C-II and apo C-III may have a similar metabolic pathway but (as in the subspecies) they may have a different pathway.

The particle sizes of our Lp A-I/A-II and Lp A-I were consistent with the findings of Cheung and Albers. 1 We found no age- and sex-related differences in the particle sizes of Lp A-I/A-II and Lp A-I. Therefore, age- and

### Table 6. Apo C-II/Apo C-III Ratio in Lipoproteins Containing Apolipoproteins A-I and A-II and In Lipoproteins Containing Apolipoprotein A-I Only

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp A-I/A-II</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td>0.31±0.03</td>
<td>0.28±0.03</td>
<td>0.30±0.02</td>
</tr>
<tr>
<td>Lp A-I</td>
<td>0.48±0.04</td>
<td>0.48±0.05</td>
<td>0.45±0.03</td>
</tr>
</tbody>
</table>

Values are mg/dl (mean±SEM). Each group comprised 10 female and 10 male subjects. a, p<0.005.
sex-related changes of Lp A-I and Lp A-I/A-II in normolipidemic subjects do not affect the particle size. Thus, a decline of Lp A-I levels during the pubertal stage in the male subjects may be due to the decreased number of Lp A-I particles.

In conclusion, when we take into account the constancy of Lp A-I/A-II levels in all groups and the higher percent of cholesterol in Lp A-I than in Lp A-I/A-II in Groups B and C males, it is likely that differences in physiological function (ability for reverse cholesterol transport) of A-I Lp (HDL) in each individual may be limited by the Lp A-I levels. Furthermore, our studies of patients with chronic renal failure and of obese subjects (both of whom are at high risk for coronary heart disease) indicate that Lp A-I was selectively decreased (Ohta T et al., unpublished observation). This evidence suggests that Lp A-I may be responsible for the sex difference in the incidence of coronary heart disease.

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References


Index Terms: apo A-I • affinity chromatography • apolipoprotein • lipoprotein
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