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. 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.

(Arteriosclerosis 9:90–95, January/February 1989)
Electrophoresis of lipoproteins isolated in 15% polyacrylamide gels containing 0.1% sodium dodecyl sulfate was performed according to the method of Weber and Osborn. 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 $0.025$).

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, choleseryl ester, and phospholipid were significantly lower in males than in females ($p<0.01$ or $0.005$). The levels of total cholesterol, choleseryl 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.
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.

### 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-I</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>197.0±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>28.8</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>

*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 Apoprotein 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>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±3.3</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>

### Table 3. Lipid and Apolipoprotein Concentrations of Lipoproteins Containing Both Apoprotein 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>
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 Apoproteins A-I and A-II and of Lipoproteins Containing Apoprotein 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 0.025). 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.

**Apoprotein 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 Apoproteins A-I and A-II and of Lipoproteins Containing Apoprotein 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
tering estrogen to premenopausal women results in a striking increase in HDL components. Our female sub-

components of A-1 Lp. It has been reported that administra-

tions on Japanese children. Similar to Caucasian

Lp components in females seem to be more complex.

Discussion

There are other reports on A-1 Lp and its subspecies, which were isolated by immunoaffinity chromatography. 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-1 Lp and Lp A-1 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. In consideration of the similarity between HDL and A-1 Lp, our lipid and apolipoprotein data of A-1 Lp in males confirmed these reports and further extended the findings to phospholipid, cholesterol, ester, and apo A-1, although the apo A-1 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 in A-1 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-1 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. Although we did not do a hormonal analysis, we did compare our results with hormonal observations on Japanese children. 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-1 Lp. It has been reported that administering estrogen to premenopausal women results in a striking increase in HDL components. Our female subjects showed no age-related changes in A-1 Lp components, despite possible increases in plasma estradiol levels. The relationships between sex hormones and A-1 Lp components in females seem to be more complex. Except for apo A-1, only apo E levels in A-1 Lp after puberty were higher in females. Williams et al. show that phospholipid liposomes acquire apo E in plasma and block cholesterol loading of cultured macrophages. Our results showing apo E in A-1 Lp may be explained by the fact that phospholipid is rich in A-1 Lp in females.

HDL2 (1.063<d<1.125), and HDL3 (1.125<d<1.21) are widely accepted as subspecies of HDL. HDL2 cholesterol is apparently higher in females than in males older than 20 years of age. In addition, the levels of HDL3 cholesterol are strongly and inversely correlated with the incidence of coronary heart disease. However, even in these lipoproteins, age- and sex-related changes in other components such as apolipoproteins, phospholipid, cholesterol, ester, and triglyceride have remained obscure. Nesstruck et al. reported that lipoproteins containing apo A-1 but no apo A-II (their fractions 1 and 2) were HDL3-like and that lipoproteins containing apo A-1 and apo A-II (their fractions 4 to 6) were HDL2-like, as determined from their hydrated densities. Atmeh et al. using immunoprecipitation procedures, found that approximately 70% of the particles in HDL2 contained apo A-1 but no apo A-II, and that 67% and 33% of these particles were found in HDL3, respectively. They also found that HDL3 comprised mainly lipoproteins containing apo A-1 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 HDL3 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 components of Lp A-I/A-II (except apo A-I) were fairly constant indicate that the characteristics of HDL2 and HDL3 reported may depend mostly on those of Lp A-I and Lp A-I/A-II, respectively.

Huff et al. 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-1 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. 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
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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
Age- and sex-related differences in lipoproteins containing apoprotein A-I.

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