Studies on the lipid and apolipoprotein compositions of two species of apoA-I-containing lipoproteins in normolipidemic males and females

Takao Ohta,[1] Shinzaburo Hattori, Soroku Nishiyama, and Ichiro Matsuda

Department of Pediatrics, Kumamoto University Medical School, Kumamoto 860, Japan

Abstract Two species of apoA-I-containing lipoproteins (A-ILp), lipoprotein containing apoA-I and apoA-II (LpA-I/A-II) and lipoprotein containing apoA-I but no apoA-II (LpA-I), have been isolated from 20 normolipidemic adults (10 males and 10 females) by immunoaffinity chromatography. We have characterized the lipid and apolipoprotein compositions in these lipoproteins, and found sex differences. In A-ILp, the levels of lipids, except triglyceride, and the level of apoE were significantly higher in females than in males. In LpA-I/A-II, sex differences were found only in the levels of apoA-I and apoE. In LpA-I, the levels of all lipids, except triglyceride, and the level of apoA-I were significantly higher in females than in males. Therefore, sex differences observed in A-ILp appear to be due primarily to the differences found in LpA-I. Of considerable significance is our finding that the ratio of cholesteryl ester to total cholesterol in LpA-I was significantly lower than that in LpA-I/A-II in both males and females. This might suggest that LpA-I could be a carrier of free cholesterol. —**Ohta, T., S. Hattori, S. Nishiyama, and I. Matsuda.** Studies on the lipid and apolipoprotein compositions of two species of apoA-I-containing lipoproteins in normolipidemic males and females. *J. Lipid Res.* 1988. **29:** 721–728.

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Many epidemiological studies have indicated that the plasma level of high density lipoprotein (HDL) is inversely correlated with the risk for coronary heart disease. Although the true mechanism involved in the inverse relationship is still under investigation, it has been proposed that HDL may play a role in "reverse cholesterol transport," i.e., the transport of cholesterol from peripheral cells to the liver for excretion in the bile (1, 2).

In earlier studies, HDL was isolated by ultracentrifugation and regarded as homogeneous particles with apoA-I and apoA-II. However, results of recent studies, using immunological techniques or rate zonal ultracentrifugation, demonstrated that HDL is heterogeneous and is comprised of subpopulations with different lipid and apolipoprotein compositions (3–7). Several research groups have identified at least two species of apoA-I-containing lipoproteins (A-ILp) after immunoaffinity chromatography; lipoprotein containing apoA-I and apoA-II (LpA-I/A-II) and lipoprotein containing apoA-I but no apoA-II (LpA-I) (4–7). These lipoproteins show HDL-like characteristics in terms of electrophoretic mobility, size, and lipid and apolipoprotein compositions. Fielding and Fielding (8, 9) suggested that the movement of cholesterol from cells into the plasma is greatly facilitated by apoA-I-containing lipoprotein unassociated with other major apolipoproteins (LpA-I with no other major apolipoproteins), and that LpA-I/A-II is involved in the esterification of free cholesterol. They have also reported that LpA-I/A-II has nothing to do with the first step of cholesterol transport from cells. Their results indicate that "reverse cholesterol transport" may be limited by the plasma concentration of LpA-I with no other major apolipoproteins.

Recently, we developed anti-apoA-I and anti-apoA-II immunoaffinity columns using a formylated derivative of cellulose gel. With this technique, we also isolated two species of apoA-I-containing lipoproteins (LpA-I/A-II and LpA-I). In the present study, we have determined the lipid and apolipoprotein compositions of LpA-I/A-II and LpA-I in normolipidemic healthy adults, and investigated sex differences.

MATERIALS AND METHODS

Isolation of antibodies monospecific for apoA-I and apoA-II

Antisera to apoA-I and apoA-II were obtained from rabbits as described previously (10). Antibodies specific for

Abbreviations: HDL, high density lipoprotein; A-ILp, lipoprotein containing apoA-I; LpA-I/A-II, lipoprotein containing apoA-I and apoA-II; LpA-I, lipoprotein containing apoA-I but no apoA-II.

[1]To whom reprint requests should be addressed.

either apoA-I or apoA-II were isolated from the respective antisera by affinity chromatography (11). Ten mg of apoA-I or apoA-II was coupled to CNBr-activated Sepharose 4B according to the procedure of the manufacturer (Pharmacia). One mg of apoA-I or apoA-II was conjugated per ml of Sepharose gel, respectively.

ApoA-I/Sepharose and apoA-II/Sepharose were transferred to chromatography columns, the final gel bed being 1.5 cm × 8.0 cm. Eighty ml of anti-apoA-I and anti-apoA-II sera were applied to the apoA-I/Sepharose and apoA-II/Sepharose column, respectively. After extensive washing with 0.01 M Tris, 0.5 M NaCl, pH 7.4, the specific bound antibodies were eluted with 0.2 M glycine/HCl, pH 2.2. These antibodies were immediately adjusted to pH 7.4 with 1.0 M Tris, and then dialyzed against 0.1 M phosphate buffer containing 0.15 M NaCl, pH 7.0, for conjugation to the Formyl-Cellufine gel.

Antibody specificity

When the anti-apoA-I antibody isolated by immunoaffinity chromatography was tested by immunodiffusion against apoA-I, A-II, B, C-II, C-III, E, HDL, human albumin, and whole plasma, it showed a single precipitin line of identity among apoA-I, HDL, and whole plasma, and did not react with the other proteins. The anti-apoA-II antibody also showed a single precipitin line of identity among apoA-II, HDL, and whole plasma, and did not react with the other proteins. Under the present conditions, the unbound fractions on the immunoaffinity columns did not react with any plasma proteins.

Preparation of anti-apoA-I and anti-apoA-II immunosorbents

Formyl-Cellufine, a formylated derivative of cellulose gel, was purchased from Seikagaku Kogyo Co, Ltd, Tokyo, Japan. Eighty mg of antibodies to apoA-I and apoA-II isolated by affinity chromatographies were incubated with 16 ml (packed gel) of Formyl-Cellufine in a final volume of 20 ml of 0.15 M NaCl and 0.1 M sodium phosphate buffer (pH 7.0). After incubation at 30°C for 30 min, sodium cyanoborohydride (60 mg) was added to reduce the Schiff base formed between antibodies and gels, followed by further incubation at 4°C for 8 hr. The gels were washed with 0.1 M sodium phosphate buffer (pH 7.0) containing 0.15 M NaCl, and then the unreacted formyl groups of the gels were blocked by incubation with 1 M monoethanolamine. The gels were finally washed and equilibrated with 0.01 M Tris, 0.5 M NaCl, 1 mM EDTA, pH 7.5 (buffer A). Under the present conditions, approximately, 92% of the antibodies was coupled to the gels.

Capacities of immunosorbent columns

Capacities of our immunosorbent columns were determined as the amount of plasma apoA-I or apoA-II bound per ml of immunosorber per single passage. Thirty ml of plasma, containing 42 mg of apoA-I and 9.6 mg of apoA-II, was applied to each immunosorbent column. Binding capacities of anti-apoA-I/Formyl-Cellufine was 0.44 mg of apoA-I per ml, and that of anti apoA-II/Formyl-Cellufine was 0.30 mg of apoA-II per ml. Nonspecific binding, measured by applying human serum albumin, was negligible in this gel system. The binding capacities did not diminish in 200 elutions.

Recoveries of apolipoproteins and lipids isolated from the immunosorbent column using Formyl-Cellufine

All elution buffers tested, such as 0.1 M or 1.0 M acetic acid, pH 3.0, 0.2 M glycine/HCl, pH 2.2, 6 M guanidine HCl, and 3 M NaScN, resulted in 85 to 87% recovery of apoA-I and apoA-II after concentration by ultrafiltration apparatus. Therefore, we employed 0.1 M acetic acid, pH 3.0, as the elution buffer. In our experiments, 85 to 87% of lipids and apolipoproteins applied was recovered in the bound fraction and unbound fraction. To determine the loss of lipids and apolipoproteins during the concentration step, we repeated this step several times. Fourteen percent of the lipids and apolipoproteins was lost during each concentration step, but there was no differential loss of lipids and apolipoproteins. Therefore, taking into account the loss of lipids and apolipoproteins during concentration, recoveries were nearly 100%. Therefore, data in Tables 2–9 were adjusted to allow for the loss during concentration of samples.

Isolation of apoA-I-containing lipoprotein by immunosorbent column using Formyl-Cellufine

Blood samples were obtained from healthy adult normolipidemic volunteers from the Kumamoto University Medical School Staff and students (ten males and ten females, between 18 and 35 years of age) after overnight fasting. The details of these subjects are summarized in **Table 1.** Informed consent was obtained from all subjects. All the study subjects consumed ordinary Japanese foods. None of the subjects were on medications or unusual diets. Venous blood was drawn into Vacutainer tubes containing disodium EDTA (1.5 mg/ml), and then the plasma was promptly separated by low speed centrifugation at 4°C.

Fresh plasma (3 ml) was applied on an anti-apoA-I immunosorbent column (1.5 × 10 cm). After washing extensively with buffer A, the column was eluted with 30 ml of 0.1 M acetic acid, 1 mM EDTA (pH 3.0) at a flow rate of 20 ml/hr. 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 apoA-I-containing lipoprotein (A-ILp)

TABLE 1. Physical data of study subjects

Subject	Sex	Age	Height	Body Weight
		yr	*cm*	*kg*
1	M	23	170	58
2	M	23	168	60
3	M	18	162	55
4	M	25	172	63
5	M	22	171	64
6	M	24	165	58
7	M	20	173	65
8	M	30	165	60
9	M	24	184	75
10	M	35	170	66
Mean		24.4	170.0	62.4
11	F	33	160	52
12	F	24	155	48
13	F	28	162	50
14	F	25	159	50
15	F	21	162	51
16	F	18	163	54
17	F	21	155	46
18	F	21	154	44
19	F	24	156	47
20	F	23	159	54
Mean		23.8	158.5	49.6

into LpA-I/A-II and LpA-I. The sample was applied on an anti-apoA-II immunosorbent column. The column was washed with buffer A to obtain the unbound fraction (LpA-I). The bound fraction (LpA-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 each was concentrated with an ultrafiltration apparatus (Amicon) to 6 ml in buffer B.

Protein and lipid analysis

The apoA-I, apoA-II, apoC-II, apoC-III, and apoE concentrations of plasma, A-ILp, LpA-I/A-II, and LpA-I were measured by radial immunodiffusion assay (10, 12). The range of standards and lower limit of sensitivity of each assay were, respectively: apoA-I, 57–228 μg/ml and 2 μg/ml; apoA-II, 14–57 μg/ml and 1 μg/ml; apoE, 14–59 μg/ml and 1 μg/ml; apoC-II, 11–47 μg/ml and 1 μg/ml; apoC-III, 27–110 μg/ml and 2 μg/ml. Therefore, appropriate concentrations were needed to measure the levels of apoC-II, C-III, and E in A-ILp, LpA-I/A-II, and LpA-I. The apoA-I and apoA-II antibodies used for radial immunodiffusion assays were different from those used for making the immunoaffinity columns. Cholesterol and triglyceride concentrations of plasma, A-ILp, LpA-I/A-II, and LpA-I were analyzed on an ABA 100 AutoAnalyzer (Abbott Laboratory) by enzymatic methods (13, 14). Cholesteryl ester was measured by the enzymatic method employing fluorometry (15). Phospholipid was analyzed by the method of Bartlett (16). The protein content of each fraction from the immunosorbent columns was determined by the method of Lowry et al. (17).

Electrophoretic analysis

Agarose gel electrophoresis was performed using a Pol-E Film system for lipoprotein electrophoresis (Corning) at pH 8.6, followed by staining with Fat Red 7B. Slab gel electrophoresis of lipoproteins in 15% polyacrylamide gels containing 0.1% SDS was performed according to the method described by Weber and Osborn (18). The Stokes diameters of the particles isolated were estimated by gradient polyacrylamide gel electrophoresis on Pharmacia precast PAA 4/30 gels according to the procedure of the manufacturer (Pharmacia). Thyroglobulin, apoferritin, catalase, lactate dehydrogenase, and bovine albumin (Pharmacia) were used as calibrating proteins. The Stokes diameters of these calibrating proteins are, respectively, 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

Student's t test was used to evaluate the data.

RESULTS

Plasma lipid and apolipoprotein levels

The plasma lipid and apolipoprotein levels are summarized in **Table 2.** The plasma triglyceride levels in males were significantly higher than those in females ($P < 0.05$). The levels of other plasma lipids and all apolipoproteins were similar in males and females. All of the plasma lipid and apolipoprotein levels in the subjects were within normal range for normal Japanese adults (12). The molar ratio of plasma apoA-I to apoA-II was slightly higher in females than in males (mean ± SEM = 2.82 ± 0.13 and 2.55 ± 0.08, respectively), but the difference was not significant.

TABLE 2. Plasma lipid and apolipoprotein concentrations measured in 10 male and 10 female subjects

	Male	Female
	mg/dl ± SEM	
Total cholesterol	185.0 ± 8.7	178.1 ± 9.8
Cholesteryl ester	132.1 ± 6.6	128.5 ± 7.2
Triglyceride	83.5 ± 11.5	54.3 ± 5.3*
Phospholipid	189.9 ± 7.5	198.4 ± 7.9
ApoA-I	136.6 ± 3.8	134.5 ± 4.7
ApoA-II	33.3 ± 1.3	30.0 ± 1.9
ApoC-II	3.61 ± 0.6	2.45 ± 0.25
ApoC-III	8.41 ± 0.86	6.90 ± 0.55
ApoE	4.42 ± 0.61	4.23 ± 0.39

*$P < 0.05$.

Lipid and apolipoprotein compositions of A-ILp

When 3 ml of plasma was applied on an anti-apoA-I immunosorbent column, all the A-ILp were removed from the plasma. The unbound fraction showed no reaction with antisera to apoA-I and apoA-II. The bound fraction (A-ILp), after elution, was found to contain apoA-I and apoA-II as major proteins on SDS-PAGE, and exhibited alpha mobility on agarose gel electrophoresis. The apolipoprotein and lipid compositions of A-ILp from the subjects are shown in **Table 3** and **Table 4.** As shown in Table 3, the total cholesterol, cholesteryl ester, phospholipid, and apoE levels in A-ILp were significantly higher in females than in males ($P < 0.05$ or 0.01 or 0.005). The triglyceride levels in A-ILp were similar in males and females. We also determined the percentages of plasma lipids and apolipoproteins. The percentages of plasma total cholesterol, cholesteryl ester, triglyceride, phospholipid, apoC-II, apoC-III, and apoE in A-ILp were significantly higher in females than in males ($P < 0.05$ or 0.02 or 0.01) (Table 4). Nearly 100% of the plasma apoA-I and apoA-II were recovered in A-ILp in both males and females. The rest of these lipids and apolipoproteins were found in the unbound fraction.

Lipid and apolipoprotein compositions of LpA-I/A-II

LpA-I/A-II, after isolation on an anti-apoA-II immunosorbent column, was found to contain both apoA-I and apoA-II on SDS-PAGE. It exhibited alpha mobility on agarose gel electrophoresis. The lipid and apolipoprotein compositions of LpA-I/A-II are shown in **Table 5** and **Table 6.** The apoA-I level was significantly lower in females than in males ($P < 0.05$). The apoE level in females was significantly higher than that in males ($P < 0.05$). The levels of other lipids and apolipoproteins were similar in females and males. The molar ratio of apoA-I to apoA-II in LpA-I/A-II was also similar in females and males (mean ± SEM = 1.56 ± 0.04 and 1.57 ± 0.08, respectively). The percentage of plasma apoA-I was significantly lower in females than in males ($P < 0.005$). The percentages of plasma apoC-III and apoE were significantly higher in females than in males ($P < 0.05$ or 0.01). The percentages of other lipids and apolipoproteins were similar in the two groups.

TABLE 3. Concentrations of lipids and apolipoproteins in A-I-containing lipoprotein (A-ILp) in 10 male and 10 female subjects

	Male	Female
	mg/dl ± SEM	
Total cholesterol	62.7 ± 3.0	76.3 ± 1.3**
Cholesteryl ester	46.6 ± 2.0	55.9 ± 0.9**
Triglyceride	18.0 ± 3.4	14.8 ± 1.3
Phospholipid	97.1 ± 4.8	122.2 ± 3.5***
ApoA-I	135.1 ± 3.0	133.5 ± 4.1
ApoA-II	33.1 ± 1.1	29.6 ± 1.9
ApoC-II	1.29 ± 0.19	1.26 ± 0.10
ApoC-III	3.91 ± 0.34	3.98 ± 0.38
ApoE	1.15 ± 0.13	1.83 ± 0.24*

*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$.

TABLE 4. Percentages of total plasma lipids and apolipoproteins in A-ILp in study subjects

	Male	Female
	% ± SEM	
Total cholesterol	34.1 ± 1.3	44.2 ± 2.6***
Cholesteryl ester	35.6 ± 1.2	45.2 ± 2.6***
Triglyceride	21.5 ± 1.7	28.1 ± 2.2*
Phospholipid	51.1 ± 1.4	60.8 ± 2.5***
ApoA-I	98.9 ± 1.3	99.2 ± 1.6
ApoA-II	99.5 ± 1.0	98.7 ± 1.1
ApoC-II	38.5 ± 3.1	54.0 ± 4.5**
ApoC-III	44.4 ± 2.8	58.3 ± 3.6***
ApoE	28.5 ± 3.3	42.4 ± 4.2**

*$P < 0.05$; **$P < 0.02$; ***$P < 0.01$.

Lipid and apolipoprotein compositions of LpA-I

LpA-I was also isolated on an anti-apoA-II immunosorbent column. LpA-I exhibited alpha mobility on agarose gel electrophoresis like LpA-I/A-II, but did not contain any apoA-II on SDS-PAGE. The lipid and apolipoprotein compositions of LpA-I from the subjects are shown in **Table 7** and **Table 8.** The total cholesterol, cholesteryl ester, phospholipid, and apoA-I levels in LpA-I were significantly higher in females than in males ($P < 0.05$ or 0.005). Other levels were similar in the two groups. The percentages of plasma total cholesterol, cholesteryl ester, phospholipid, apoA-I, apoC-II, and apoC-III in LpA-I were significantly higher in females than in males ($P < 0.01$ or 0.005). The percentages of plasma triglyceride and apoE in LpA-I were similar in the two groups.

Percent lipid compositions of A-ILp, LpA-I/A-II, and LpA-I

As shown in **Table 9,** the percentages of total cholesterol, phospholipid, and triglyceride in A-ILp and LpA-I/A-II were similar in the two groups. The ratios of cholesteryl ester to total cholesterol in A-ILp, LpA-I/A-II, and LpA-I were also similar in the two groups. The percentage of phospholipid in LpA-I was significantly higher in females than in males ($P < 0.005$). The percentage of triglyceride in LpA-I was significantly lower in females than in males ($P < 0.05$). When the lipid composition of LpA-I was comapred with that of LpA-I/A-II, the percentage of LpA-I phospholipid in males was found to be significantly lower, and the ratio of cholesteryl ester to total cholesterol in LpA-I in both groups was also significantly lower than that in LpA-I/A-II ($P < 0.005$).

TABLE 5. Concentrations of lipids and apolipoproteins in LpA-I/A-II in 10 male and 10 female subjects

	Male	Female
	mg/dl ± SEM	
Total cholesterol	38.7 ± 1.2	40.4 ± 1.3
Cholesteryl ester	29.4 ± 0.9	30.5 ± 1.0
Triglyceride	9.29 ± 1.67	7.52 ± 0.82
Phospholipid	63.0 ± 1.9	68.9 ± 3.7
ApoA-I	84.2 ± 3.0	75.0 ± 2.9*
ApoA-II	32.5 ± 1.1	29.8 ± 1.2
ApoC-II	0.70 ± 0.11	0.50 ± 0.06
ApoC-III	2.33 ± 0.16	2.22 ± 0.20
ApoE	0.76 ± 0.06	1.37 ± 0.21*

*$P < 0.05$.

Particle diameter of LpA-I/A-II and LpA-I

LpA-I exhibited two distinct particle sizes, mean Stokes diameter 11.1 nm and 8.8 nm. LpA-I/A-II exhibited three distinct particle sizes, mean Stokes diameter 10.1 nm, 9.0 nm, and 8.1 nm. The Stokes diameters of these particles were similar in both males and females.

DISCUSSION

HDL has been well recognized as a protective factor for atherosclerotic cardiovascular disorders. Structurally, HDL was previously treated as homogeneous particles consisting of the same lipids and apolipoproteins. However, recent studies have shown that HDL is a complex of particles with different hydrated densities, such as HDL_2 and HDL_3, and different apolipoprotein compositions (3–7). Many reports have indicated that the plasma concentration of HDL_2 is strongly and inversely correlated with the incidence of coronary heart disease. HDL_2 is higher in females, who are generally resistant to coronary heart diseases, than in males at all ages (19, 20). As to subpopulations with different protein compositions, only a few reports have appeared (5–7). Alterations in these particles have been less well investigated, even in normolipidemic adults.

In the present study, we isolated LpA-I/A-II and LpA-I from 20 normolipidemic subjects (10 men and 10 women) by immunoaffinity chromatography using antibody monospecific for apoA-I and apoA-II. These lipoproteins exhibit a mobility on agarose gel electrophoresis similar to that of HDL isolated by conventional ultracentrifugation. When these lipoproteins were removed from plasma by the immunoaffinity chromatography, no α-migrating lipoprotein was detected, suggesting that LpA-I/A-II and LpA-I account for most of the α-lipoprotein (HDL).

In our subjects, the plasma lipid and apolipoprotein levels were similar in males and females except for the plasma triglyceride level, which was higher in males than in females. This finding was consistent with the results of another study on a large Japanese population (12).

A-ILp has also been isolated by several investigators, but they did not mention lipid and apolipoprotein levels other than those of apoA-I, apoA-II, and apoE. In the present study, the total cholesterol, cholesteryl ester, phospholipid, and apoE levels in A-ILp were found to be higher in females than in males. The cholesterol levels were 10 to 15 mg/dl higher than that of HDL-cholesterol determined by the precipitation method in both groups (males, 54.5 mg/dl; females, 58.8 mg/dl). This suggests two possibilities. First, loss of cholesterol during the precipitation procedure, and second, contamination of chylomicron in these fractions because apoA-I is one of the apoprotein components. However, the latter possibility is unlikely because no chylomicron was detected on agarose gel electrophoresis. According to the report by Stein, Vanderhoek, and Stein (21), phospholipid-enriched HDL is able to promote cholesterol efflux from fibroblasts. If this is so, the finding of phospholipid-rich A-ILp in females could be compatible with the epidemiological data described above. The percentages of plasma lipids and apolipoproteins in A-ILp other than apoA-I and apoA-II were also higher in females than in males, although no sex differences were found in the levels of apoC-II, apoC-III, and triglyceride. At this stage, it is not known whether the concentration or the percentage of the plasma level is more important for studying the function of A-ILp. In the case of plasma apoE, Cheung and Albers (5) found that 69% of plasma apoE was associated with apoA-I. The present data are quite different; only 29% (males) and 42.4% (females) of the plasma apoE was found to be associated with apoA-I. There is no obvious reason for this discrepancy, but it is likely due to the method used for the isolation of A-ILp. Another possibility is raised by the recent report that the distribution of plasma apoE in normolipidemic subjects is quite variable (22). Thus this discrepancy might contribute, to some

TABLE 6. Percentages of total plasma lipids and apolipoproteins in LpA-I/A-II in study subjects

	Male	Female
	% ± SEM	
Total cholesterol	21.2 ± 0.7	23.2 ± 1.3
Cholesteryl ester	22.6 ± 0.8	24.6 ± 1.4
Triglyceride	11.3 ± 1.0	14.3 ± 1.5
Phospholipid	33.4 ± 1.0	34.1 ± 1.6
ApoA-I	63.1 ± 1.0	55.8 ± 1.3***
ApoA-II	97.6 ± 1.1	99.2 ± 1.2
ApoC-II	21.1 ± 1.8	20.5 ± 1.2
ApoC-III	26.7 ± 1.5	32.3 ± 1.8*
ApoE	19.2 ± 2.2	31.4 ± 3.6**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$.

TABLE 7. Concentrations of lipids and apolipoproteins in LpA-I in 10 male and 10 female subjects

	Male	Female
	mg/dl ± SEM	
Total cholesterol	24.0 ± 1.9	35.9 ± 1.4**
Cholesteryl ester	17.2 ± 1.3	25.3 ± 0.9**
Triglyceride	8.7 ± 1.8	7.1 ± 0.8
Phospholipid	34.1 ± 3.4	55.9 ± 2.3**
ApoA-I	50.7 ± 2.1	59.6 ± 3.0*
ApoA-II	0 ± 0	0 ± 0
ApoC-II	0.56 ± 0.10	0.76 ± 0.06
ApoC-III	1.58 ± 0.22	1.78 ± 0.19
ApoE	0.39 ± 0.08	0.46 ± 0.07

*$P < 0.05$; **$P < 0.005$.

extent, to the difference in apoE distribution among the subjects.

To study the subpopulations of A-ILp, we isolated LpA-I/A-II and LpA-I from A-ILp on an anti-apoA-II immunosorbent column. Similar attempts have been made by several groups. From hydrated densities, Nestruck et al. (7) reported that LpA-I resembles HDL_2 and LpA-I/A-II resembles HDL_3. According to Atmeh, Shepherd, and Packard (6), about 70% of HDL_2 particles were LpA-I, and two-thirds of LpA-I was found in HDL_2 and the remainder in HDL_3. LpA-I/A-II represented most of the HDL_3 particles, although LpA-I was a minor component. These data taken together suggest the close relationship between HDL_2 and LpA-I, and between HDL_3 and LpA-I/A-II. However, these studies do not shed light on the alterations in the plasma concentrations of LpA-I/A-II and LpA-I, even in normolipidemic subjects.

As to LpA-I/A-II, sex differences were found in the levels of apoA-I and apoE, and in the percentages of plasma apoA-I, apoC-III, and apoE (Tables 5 and 6). Atmeh et al. (5) and Cheung and Albers (6) previously reported that about 55% to 62% of plasma apoA-I was associated with LpA-I/A-II. This is consistent with our findings. The molar ratio of apoA-I to apoA-II in LpA-I/A-II was quite constant in both males and females, regardless of the percentage of LpA-I. This finding is also consistent with previous reports (5, 6). Most of the apoE associated with apoA-I was found in LpA-I/A-II in both groups. Similar findings have been reported by Cheung and Albers (5).

As to LpA-I, the concentrations as well as percentage of its major components such as total cholesterol, cholesteryl ester, phospholipid, and apoA-I were higher in females than in males (Tables 7 and 8). In addition, the percentages of plasma apoC-II and apoC-III were also higher in females. Based on these results, the sex differences observed in A-ILp appear to be primarily due to the differences found in LpA-I.

Cheung and Albers (5) did not find a difference in lipid compositions between LpA-I/A-II and LpA-I. However, the present results showed that the percentage of triglyceride in LpA-I was lower, and the percentage of phospholipid was higher in females than in males (Table 9). Of considerable significance is our finding that the lipid ratio of cholesteryl ester to total cholesterol in LpA-I was significantly lower than that in LpA-I/A-II in both males and females. In this connection, from the experiments using LpA-I with no other major apolipoprotein and LpA-I/A-II, Fielding and Fielding (8, 9) have suggested that *i*) both apoD and lecithin:cholesterol acyltransferase (LCAT) are necessary for the esterification of free cholesterol even in the presence of apoA-I; *ii*) most of LCAT is present in the LpA-I fraction, whereas LpA-I/A-II possesses ~80% of the plasma apoD; and *iii*) LpA-I plays a major role in the first step of the transport process of cellular cholesterol, although the esterification of cholesterol does necessitate both LpA-I/A-II and LpA-I. LpA-I with no other major apolipoproteins could be a carrier of free cholesterol. Unlike the LpA-I described by Fielding and Fielding (8, 9), our LpA-I was isolated using an anti-apoA-I and anti-apoA-II immunosorbent column, and contained apoE and apoCs. Based on their data, the plasma concentration of apoA-I unassociated with other major apolipoproteins was 95 ± 10 μg/ml. Plasma concentration of apoA-I in our LpA-I was 500–600 μg/ml. These results indicate that only 16–19% of our LpA-I is equivalent to the LpA-I of Fielding and Fielding (8, 9). However, their anti-apoA-II column removed ~95% of apoA-I from plasma. This is very different from our present data and the data of Cheung and Albers (5) (approximately 55–60%). According to the recent report by Cheung et al. (23), 70–80% of LCAT and cholesteryl ester transfer activity was recovered in apoA-I-containing lipoproteins without apoA-II. (This lipoprotein has properties similar to our LpA-I.) Therefore, it seems likely that Fielding and Fielding may have underestimated the plasma concentration of their LpA-I. If this

TABLE 8. Percentages of total plasma lipids and apolipoproteins in LpA-I in study subjects

	Male	Female
	% ± SEM	
Total cholesterol	12.9 ± 0.9	21.0 ± 1.6***
Cholesteryl ester	13.0 ± 0.8	20.6 ± 1.5***
Triglyceride	10.2 ± 0.9	13.5 ± 1.2
Phospholipid	17.7 ± 1.2	27.9 ± 1.6***
ApoA-I	36.9 ± 1.0	44.3 ± 1.4***
ApoA-II	0 ± 0	0 ± 0
ApoC-II	17.4 ± 2.4	33.6 ± 4.2***
ApoC-III	17.6 ± 2.0	25.7 ± 1.9**
ApoE	9.3 ± 1.6	11.0 ± 1.8

**$P < 0.01$; ***$P < 0.005$.

TABLE 9. Lipid composition of A-ILp, LpA-I/A-II, and LpA-I in study subjects

		Total Cholesterol	Phospholipid	Triglyceride	$\frac{\text{Cholesteryl Ester}}{\text{Total Cholesterol}} \times 100$
		% of total lipid ± SEM			
A-ILp	M	35.4 ± 0.6	54.8 ± 0.9	9.8 ± 1.5	74.6 ± 0.7
A-ILp	F	35.8 ± 0.5	57.3 ± 0.7	6.9 ± 0.6	73.3 ± 0.4
LpA-I/A-II	M	34.9 ± 0.5	56.9 ± 0.9[a]	8.3 ± 1.3	76.1 ± 0.6[d]
LpA-I/A-II	F	34.8 ± 0.6	58.9 ± 0.9	6.4 ± 0.6	75.7 ± 0.4[e]
LpA-I	M	36.3 ± 0.9	51.1 ± 1.0[a,b]	12.6 ± 1.8[c]	72.1 ± 0.9[d]
LpA-I	F	36.3 ± 0.7	56.6 ± 1.0[b]	7.2 ± 0.7[c]	70.8 ± 0.5[e]

Differences between values with same letter: [a,b,d,e], $P < 0.005$; [c], $P < 0.05$.

is so, it is reasonable that the ratio of cholesteryl ester to total cholesterol in our LpA-I is significantly lower than that in LpA-I/A-II.

When we consider "reverse cholesterol transport" as a function of HDL, we have to explain the discrepancy between the results of epidemiological studies (19, 20) and experimental in vitro studies (24, 25). Namely, many epidemiological studies have indicated that the plasma levels of HDL_2 are inversely correlated with the incidence of ischemic heart disease, but in vitro experimental studies have indicated that only HDL_3 is involved in the cholesterol efflux from peripheral cells, whereas HDL_2 has the opposite effect on this process. This discrepancy might be explained by the recent reports by Cheung et al. (5, 23) indicating that LCAT and cholesteryl ester transfer protein are lost from HDL_2 and HDL_3 during ultracentrifugation (5, 23). This might have some influence on in vitro behavior of HDL_2 and HDL_3.

If our speculation mentioned above is true, the in vitro data of Fielding and Fielding (8, 9) seem to be consistent with those of the present clinical study; LpA-I is higher in females than in males. If so, the level of LpA-I may be a more reliable predictor for the risk of coronary heart disease than the levels of HDL_2, although further studies on high risk groups for ischemic heart diseases are needed to clarify this concept.

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