Composition of Plasma ApoA-I-Containing Lipoprotein Particles in Children and Adults

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ABSTRACT. The purpose of this study was to examine sex- and age-related differences in the concentration and composition of lipoprotein particles containing apoA-I (LP-A-I) and those containing apoA-I and apoA-II (LP-A-I:A-II), the main HDL as defined by their apolipoprotein composition. Lipoproteins were isolated by immunoaffinity chromatography of whole plasma from 16 normal prepubertal children and 15 normal male and female adults using "pan"-MAB to apoA-I and apoA-II. Although there was no difference between children and adults in the concentration of LP-A-I:A-II, adult females had significantly higher levels of LP-A-I than either children or adult males. Main differences between children and adults as well as between adult males and females were in the apolipoprotein composition of the lipoprotein particles; children had the highest content of minor apolipoproteins (apoC and apoE) in LP-A-I but the lowest in LP-A-I:A-II. The lipid/apolipoprotein ratios of LP-A-I and LP-A-I:A-II were significantly higher in children and women than in men. The LP-A-I and LP-A-I:A-II contained 75% of the total plasma apoC and apoE in women and children but only 50% in men. However, in all three groups, 70–90% of the minor HDL apolipoproteins were associated with LP-A-I:A-II. The nonmolar ratios of minor apolipoproteins in LP-A-I and LP-A-I:A-II and the sex- and age-related differences in apoA-I/apoA-II ratios of LP-A-I:A-II suggest that both lipoproteins may consist of a spectrum of lipoprotein subfamilies differing in their apolipoprotein composition. Results of this study indicate that the age- and sex-related differences in the levels and composition of LP-A-I and LP-A-I:A-II relate primarily to changes in the protein moieties of these particles. (*Pediatr Res* 29: 315–321, 1991)

Abbreviations

LP-A-I, lipoprotein particle containing apoA-I
LP-A-I:A-II, lipoprotein particle containing apoA-I and apoA-II
HDL-C, HDL-cholesterol
TG, triglyceride
PL, phospholipid
CE, cholesterol ester
FC, free cholesterol

Previous evidence clearly supports heterogeneity of operationally defined plasma lipoprotein classes with respect to their chemical composition and metabolic properties (1–3). For example, by utilizing apolipoproteins as specific markers for identifying and classifying discrete lipoprotein particles in plasma, it has been shown that apoB- and apoA (apoA-I and apoA-II)-containing lipoproteins form two major groups of plasma lipoproteins (1, 2). The apoB-containing lipoproteins are located in the very low and low density ranges, whereas the apoA-containing lipoproteins are present in the high and very high density ranges of the lipoprotein density spectrum. The latter group consists of two major lipoprotein families referred to on the basis of their characteristic apolipoprotein composition as LP-A-I:A-II and LP-A-I. The isolation and partial characterization of these two lipoprotein families have been described in reports from several laboratories (4–11).

So far, the LP-A-I:A-II and LP-A-I particles have been isolated mainly from plasma of normolipidemic adults. However, because the human lipid transport system has been shown to undergo several changes from its fetal to adult form (2, 12–15), the purpose of this study was to provide information on the concentration and composition of these two major HDL families in plasma of normal, prepubertal children of both sexes and to compare them with those of adults.

SUBJECTS

A group of 16 normolipidemic children (10 males and six females), aged 4 to 9 y, were randomly selected from patients admitted to the outpatient surgical facility of the Oklahoma Children's Memorial Hospital for minor surgical procedures. All children were healthy, asymptomatic, and without family history of hyperlipoproteinemia or diabetes; they had normal weight and height for their age groups. The conditions under which the blood samples were drawn were unstressful. There was minimal anticipation of the event; it took place soon after the parents, waiting with their children in a playroom, had given their written consent. Blood samples were collected into vacutainer tubes containing disodium EDTA (1.5 mg/mL) and the plasma was promptly separated by low centrifugation (1000 × g) at 4°C.

The adult population consisted of seven male and eight female volunteers randomly selected from a pool of normolipidemic employees of the Oklahoma Medical Research Foundation according to some predetermined selection criteria (2). All subjects were healthy, asymptomatic Caucasians with no history of familial hyperlipoproteinemia or diabetes. Their weight/height index was between 0.9 and 1. They were all nonsmokers except for one of the male subjects who consumed 10 cigarettes/d. Only two male subjects were regular alcohol drinkers, but their alcohol consumption did not exceed 50 g/wk. All women were premenopausal and none was using contraceptive agents. With the exception of one male subject, age 64 y, the mean age of men

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(34.8 ± 6.7 y) was very similar to that of women (35.6 ± 8.5 y). Informed consent was obtained from each adult volunteer. After an overnight fast, blood samples were drawn and plasma samples were prepared as already described for children.

MATERIALS AND METHODS

Production and characterization of MAb to apoA-I and apoA-II. MAb F59 4A12 2F4 (anti-apoA-I) and CdB₅ (anti-apoA-II) were produced, purified, and characterized as previously described (16, 17). These antibodies were selected on the basis of their specificity and binding capacity to the major circulating forms of corresponding apolipoproteins.

Preparation of anti-apoA-I and anti-apoA-II immunosorbers. MAb to apoA-I and apoA-II were coupled to the cross-linked agarose activated with N-hydroxysuccinimide (Affi-Gel 10) purchased from Bio-Rad Laboratories (Richmond, CA). After exhaustive washing of Affi-Gel with ice-cold, double-distilled, deionized water, the solubilized antibodies were added to the gel slurry (0.8 mg of Ig/mL of gel) and the mixture was gently shaken for 2 h at room temperature. The gel was allowed to settle and the supernatant solution was carefully removed. To block the remaining active sites, the gel was incubated overnight with 0.5 M Tris-HCl buffer containing 1.5 M NaCl, pH 7.4, at 4°C. The Econo-columns (1.5 × 30 cm; Bio-Rad Laboratories) were first packed with 30 mL of Sephadex G-25 (Pharmacia, Piscataway, NJ) followed by 20 mL of antibody-coupled Affi-Gel and another protective layer of 2.5 mL of Sephadex G-25. The purpose of Sephadex G-25 layers was to shorten the exposure time between the lipoprotein and dissociating agent (18). Each column was extensively washed free of nonadsorbed proteins with three volumes of 3 M sodium thiocyanate and reequilibrated with 0.5 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and 1.5 g/L EDTA. The binding capacities of the anti-apoA-I and anti-apoA-II immunosorbers were 0.018 mg of apoA-I and 0.027 mg of apoA-II/mL of gel, respectively. The antibody-coupled immunosorbers were stable for more than six mo at 4°C without loss of binding capacity.

Isolation and fractionation of apoA-I-containing lipoproteins by immunoaffinity chromatography. Fresh plasma (2 mL) was applied to the anti-apoA-I immunosorber and incubated overnight at 4°C. The unretained fraction was washed off with 0.05 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and 1.5 g/L EDTA. ApoA-I-containing lipoproteins bound to the column were then desorbed and eluted with 3 M sodium thiocyanate at a flow rate of 30 mL/h. The retained fraction was concentrated in dialysis bags using polyvinyl-pyrrolidone PVP-40 (Sigma Chemical Co., St. Louis, MO) outside the bags, then applied to the anti-apoA-II immunosorber without overloading. Both the unretained (LP-A-I) and the retained (LP-A-I:A-II) fractions were eluted and concentrated as described above. The unretained fraction was tested for the possible presence of apoA-II by electroimmunoassay. Fractions containing immunochemically detectable apoA-II were rechromatographed on anti-apoA-II immunosorber and only unretained fractions free of apoA-II were used for measuring the distribution of apoA-I between retained (LP-A-I:A-II) and unretained (LP-A-I) fractions. The recoveries of apoA-I ranged between 78 and 92% of applied apoA-I. The concentrations of apoA-I in LP-A-I and LP-A-I:A-II particles were calculated on the basis of plasma apoA-I values and the percent distribution of apoA-I in retained and unretained fractions from anti-apoA-II immunosorber.

Apolipoprotein and lipid analyses. Quantitative determination of apoA-I, -A-II, -B, -C-II, -C-III, -D, and -E in plasma and in LP-A-I and LP-A-I:A-II was carried out by previously described electroimmunoassays (19–24). Total cholesterol and TG in the plasma were determined by enzymatic procedures as previously described (25). HDL-C was estimated according to the procedure of Lipid Research Clinics Program (26). These three assays were standardized with serum calibrators and control samples supplied

by the Centers for Disease Control, Atlanta, GA. TG, CE, and FC of lipoprotein particles and CE and FC in whole plasma were quantified by a gas-liquid chromatographic procedure of Kuksis *et al.* (27). PL phosphorus content was determined by the method of Gerlach and Deuticke (28).

Statistical evaluation. All values, where appropriate, were expressed as mean $\pm$ SD. Statistical differences were assessed by the *t* test. The level of significance was regarded as $p < 0.05$.

RESULTS

Plasma lipid and apolipoprotein levels. The plasma lipid and apolipoprotein concentrations of the normolipidemic children and adults are shown in Tables 1 and 2. There were no significant differences in the lipid profiles between the male and female children. However, the concentration of total cholesterol was significantly lower in children of both sexes than in adults. The plasma TG levels were significantly lower in male children but not in female children in comparison with corresponding adults. As a group, children also had significantly lower plasma PL levels than adults. Although men and women had significantly higher levels of HDL-C than male and female children, these differences were not statistically significant. The values of the CE/FC ratio of whole plasma were within the normal range in children and adults of both sexes. There were no differences in the concentrations of apoA-I, -A-II, -B, and -D between male and female children. However, despite almost identical levels of TG, female children had significantly higher levels of apoC-II, apoC-III, and apoE than male children. In contrast, the concentrations of apoC-II and -C-III, but not apoE, were significantly lower in adult females than males; women also had significantly higher concentrations of apoA-I and lower concentrations of apoA-II and apoB than men. As a group, children had significantly lower concentrations of all apolipoproteins, with the exception of apoE, than the adult population.

Plasma levels of apolipoprotein A-I associated with LP-A-I and LP-A-I:A-II. The levels of apoA-I were determined in the fractions containing LP-A-I and LP-A-I:A-II after concentrating with PVP-40. Because there was no difference between male and female children in the concentrations of apoA-I associated with either LP-A-I or LP-A-I:A-II, these results were combined and children were treated as a single group (Table 3). The apoA-I concentrations associated with LP-A-I in adult females were significantly higher than those in adult males and children ($p < 0.01$). In contrast, there was no significant difference between adult males and children in the levels of apoA-I associated with LP-A-I. When LP-A-I concentrations were expressed in terms of total lipid and protein mass, the LP-A-I levels in adult females (148.6 ± 31.2 mg/dL) were still significantly higher ($p < 0.05$ versus children and $p < 0.01$ versus adult males) than in children (121.0 ± 20 mg/dL) or adult males (92.8 ± 30.4 mg/dL). However, because the lipid but not the protein mass of LP-A-I was higher in children than in adult males, the levels of LP-A-I particles expressed in terms of lipid and protein mass were higher in children ($p < 0.05$) than in men. All three groups of normolipidemic subjects had similar concentrations of apoA-I associated with LP-A-I:A-II and similar levels of LP-A-I:A-II expressed in terms of their lipid and protein mass (348 ± 75 , 340 ± 46 , and 384 ± 54 mg/dL for children, males, and females, respectively). It appears, therefore, that significantly higher values of plasma apoA-I in women ($p < 0.05$) are mainly accounted for by the higher concentrations of apoA-I associated with LP-A-I particles.

Percent lipid compositions of LP-A-I and LP-A-I:A-II. As shown in Table 4, there were no significant differences in the percent lipid composition of LP-A-I and LP-A-I:A-II isolated from children as a single group and adult males and females as separate groups. However, the percentage of TG in LP-A-I was significantly higher in men than in women ($p < 0.05$). When the percent lipid composition of LP-A-I was compared with that of

Table 1. Plasma lipid levels (mmol/L) in normolipidemic children and adults*

Subjects	TC	TG	PL	HDL-C	CE/FC
Children					
Male (n = 10)	3.70 ± 0.65 ^c	0.50 ± 0.21 ^c	2.80 ± 0.41	1.28 ± 0.32	4.0 ± 0.33
Female (n = 6)	3.63 ± 0.73 ^d	0.53 ± 0.28	2.81 ± 0.39	1.32 ± 0.37	3.6 ± 0.77
Total (n = 16)	3.67 ± 0.66 ^f	0.51 ± 0.23	2.81 ± 0.40 ^b	1.30 ± 0.37	3.9 ± 0.56
Adults					
Male (n = 7)	5.30 ± 1.11 ^c	1.14 ± 0.58 ^{a,c}	3.80 ± 0.55	1.34 ± 0.24	3.9 ± 0.45
Female (n = 8)	5.35 ± 0.89 ^d	0.56 ± 0.16 ^a	3.70 ± 0.30	1.57 ± 0.24	4.1 ± 0.47
Total (n = 15)	5.33 ± 0.93 ^f	0.78 ± 0.46	3.75 ± 0.40 ^b	1.48 ± 0.26	4.0 ± 0.46

* Mean ± SD; all values are expressed as mmol/L except for CE/FC, which is a weight ratio. TC, total cholesterol. Means marked with the same letter are significantly different at: ^{a,b} $p < 0.05$; ^{c,d,e} $p < 0.01$; ^f $p < 0.001$.

Table 2. Plasma apolipoprotein levels in normolipidemic children and adults*

Subjects	Apolipoproteins (mg/dL)						
	A-I	A-II	B	C-II	C-III	D	E
Children							
Male (n = 10)	126.2 (14.8)	70.9 (8.9)	67.5 (15.0)	1.6 ^c (0.4)	4.6 ^a (1.5)	7.9 (1.9)	9.3 ^d (2.6)
Female (n = 6)	128.9 (21.9)	72.8 (10.6)	68.0 (13.2)	2.6 ^c (0.7)	6.1 ^a (1.5)	8.6 (2.0)	12.9 ^d (3.3)
Total (n = 16)	127.2 ^f (17.0)	71.6 ^b (9.2)	67.7 ^f (13.9)	2.0 ^b (0.7)	5.1 ^m (1.4)	8.2 ⁿ (1.9)	10.7 (3.3)
Adults							
Male (n = 7)	133.5 ^c (14.5)	90.4 ^e (10.6)	109.4 ⁱ (20.0)	3.4 ^k (1.2)	10.7 ^j (2.4)	15.9 (4.6)	11.8 (3.6)
Female (n = 8)	151.7 ^c (13.8)	76.5 ^e (4.8)	77.4 ⁱ (13.4)	2.1 ^k (0.7)	7.8 ^j (1.3)	14.4 (2.5)	11.2 (2.6)
Total (n = 15)	143.2 ^f (16.5)	82.2 ^b (10.3)	92.3 ^j (23.1)	2.7 ^b (1.1)	9.1 ^m (2.4)	15.2 ⁿ (3.7)	11.5 (3.0)

* Values are mean ± (SD). Means marked with the same letter are significantly different at: ^{a,h} $p < 0.05$; ^{c,d,e,f,g,h,i,j,k,l} $p < 0.01$; ^{m,n} $p < 0.001$.

Table 3. Plasma levels of apoA-I associated with LP-A-I and LP-A-I:A-II particles*

Subjects	LP-A-I		LP-A-I:A-II	
	mg/dL	% of total apoA-I	mg/dL	% of total apoA-I
Children (n = 16)	38.2 ± 7.8	30.2 ± 5.5	89.0 ± 15.8	69.8 ± 5.5
Adult males (n = 7)	38.5 ± 11.1	28.9 ± 6.2	93.0 ± 9.3	71.1 ± 6.2
Adult females (n = 8)	54.4 ± 11.0 [†]	35.6 ± 4.6	97.3 ± 7.4	64.4 ± 4.6

* Values are mean ± SD.

[†] Mean significantly different ($p < 0.01$) from those of adult males and children.

Table 4. Percent lipid composition of LP-A-I and LP-A-I:A-II particles*

Subjects	TG	CE	FC	PL
LP-A-I				
Children (n = 16)	3.8 ± 2.9	25.5 ± 4.4	6.7 ± 1.4	64.0 ± 5.6
Male adults (n = 7)	4.5 ± 1.8 ^a	26.5 ± 4.5	5.9 ± 1.2	63.1 ± 3.8
Female adults (n = 8)	2.1 ± 1.1 ^a	24.9 ± 4.1	7.7 ± 1.9	65.3 ± 4.8
LP-A-I:A-II				
Children (n = 16)	4.0 ± 2.2	30.2 ± 2.4	5.5 ± 1.1	60.3 ± 3.4
Male adults (n = 7)	3.9 ± 1.1	32.4 ± 2.8	5.5 ± 0.5	58.1 ± 3.9
Female adults (n = 8)	3.4 ± 1.2	31.6 ± 6.2	6.6 ± 1.3	58.4 ± 6.5

* Values are mean ± SD. Means marked with the same letter are significantly different at: ^a $p < 0.05$.

LP-A-I:A-II, the mean values for CE/FC ratios were always higher in LP-A-I:A-II (adults: 5.3 ± 12 ; children: 5.6 ± 1.0) than in LP-A-I from the same populations (adults: 3.9 ± 1.3 ; children: 3.8 ± 0.8). The higher CE/FC ratio in LP-A-I:A-II reflected a higher content of CE at the expense of PL content and, to a lesser extent, at the expense of FC content when compared with LP-A-I.

Percent apolipoprotein compositions of LP-A-I and LP-A-I:A-II. The percent apolipoprotein composition of LP-A-I and LP-A-I:A-II was affected by both the sex and age of normolipidemic subjects (Table 5 and 6). There are, however, some common characteristics regarding the apolipoprotein composition of these

lipoprotein families. First, apoA-I in LP-A-I and apoA-I and apoA-II in LP-A-I:A-II account for approximately 85% of the total protein content of these particles. Second, in both lipoprotein families regardless of sex and age, apoC-II and apoC-III account for approximately 5% and apoD and apoE for approximately 10% of their total protein content.

The LP-A-I particles from female adults had a significantly lower percentage content of apoC-III and -E ($p < 0.01$ and $p < 0.05$, respectively) and a higher percentage content of apoA-I ($p < 0.05$) than those from young subjects. However, there were no significant differences in the apolipoprotein composition of LP-A-I between children and adult males. The LP-A-I:A-II particles

Table 5. Percent apolipoprotein composition of LP-A-I particles*

Subjects	Apolipoproteins				
	A-I	C-II	C-III	D	E
Children (n = 16)	85.1 ± 5.6 ^a	1.3 ± 1.4	4.8 ± 2.4 ^b	5.0 ± 2.9	3.8 ± 2.9 ^c
Male adults (n = 7)	88.0 ± 7.8	0.4 ± 0.4	2.8 ± 3.7	5.6 ± 2.8	3.2 ± 2.8
Female adults (n = 8)	91.0 ± 6.9 ^a	0.7 ± 0.7	1.9 ± 1.5 ^b	5.2 ± 4.8	1.1 ± 1.7 ^c

* Values are mean ± SD. Means marked with the same letter are significantly different at: ^{a,c} $p < 0.05$; ^b $p < 0.01$.

Table 6. Percent apolipoprotein composition of LP-A-I:A-II particles*

Subjects	Apolipoproteins					
	A-I	A-II	C-II	C-III	D	E
Children (n = 16)	52.6 ± 3.0 ^a	34.6 ± 2.3 ^c	1.1 ± 1.2	1.4 ± 0.7 ^e	4.0 ± 1.2 ^f	4.5 ± 2.2 ^g
Male adults (n = 7)	49.3 ± 2.2 ^{a,b}	38.3 ± 2.3 ^d	0.8 ± 0.4	2.1 ± 2.4	7.1 ± 1.4 ^f	2.4 ± 0.7 ^{g,h}
Female adults (n = 8)	52.8 ± 4.7 ^b	32.9 ± 4.8 ^{c,d}	1.0 ± 0.5	2.6 ± 2.2 ^e	1.1 ± 1.7 ^c	5.5 ± 3.1 ^h

* Values are mean ± SD. Means marked with the same letter are significantly different at: ^{a,b,c,d,g,h} $p < 0.05$; ^e $p < 0.01$; ^f $p < 0.0001$.

from adult males had significantly lower relative contents of apoA-I and apoE ($p < 0.05$) and a higher percentage of apoD ($p < 0.001$) than those from children or adult females.

The molar ratio of apoA-I to apoA-II in LP-A-I:A-II particles from adult females (1.02 ± 0.18) was higher than that in LP-A-I:A-II particles from prepubertal children (0.9 ± 0.08) or adult males (0.8 ± 0.7).

Distribution of minor apolipoproteins among apoA-I-containing lipoprotein particles. In children and adult females, more than 75% of the total plasma apoC-II, apoC-III, and apoE contents were associated with apoA-I-containing lipoprotein particles. In contrast, only 50% of these apolipoproteins were associated with apoA-I-containing lipoprotein particles in plasma of normolipidemic adult males. Approximately 88% of the total plasma apoD was bound to apoA-I-containing lipoproteins.

Results in Table 7 show that, of the total content of minor apolipoproteins associated with apoA-I, approximately 75% were bound to LP-A-I:A-II and 25% to LP-A-I. However, there were some sex- and age-related differences in the distribution of these apolipoproteins. For example, in plasma of prepubertal children, about 50% of the total apoC-III associated with apoA-I was detected in LP-A-I. In plasma of adult females, more than 90% of the total apoE associated with apoA-I was bound to LP-A-I:A-II, whereas in plasma of adult males 90% of the total apoC-II was bound to LP-A-I:A-II. Men also had a higher proportion of apoD in LP-A-I:A-II (85%) than women or children.

Lipid/apolipoprotein ratios of LP-A-I and LP-A-I:A-II. The apolipoprotein contents of LP-A-I and LP-A-I:A-II are expressed as the sum of all separately measured apolipoproteins. In general, the lipid/apolipoprotein ratios of LP-A-I were higher than those of LP-A-I:A-II in all subjects (Table 8). However, the LP-A-I from men contained less lipids than those from women or children. Similarly, the relative lipid content of LP-A-I:A-II from adult males was lower than that of either adult females or children. However, there were no significant differences in lipid/

apolipoprotein ratios of LP-A-I and LP-A-I:A-II between children and women.

DISCUSSION

The well-documented negative correlation between the concentration of HDL or HDL-C and coronary artery disease (29–33) has been one of the main reasons for continuing interest in the chemical and metabolic properties of this group of lipoproteins. Studies on the chemical and immunologic characterization have shown that HDL represent a heterogeneous population of particles differing not only in hydrated density and size but also in lipid and apolipoprotein composition (1, 34, 35). This marked heterogeneity of HDL is mainly due to the well-documented occurrence of distinct, polydisperse families of lipoprotein particles of similar physical properties but different and specific apolipoprotein composition (1, 34–37). Albers and Aladjem (4) were the first to identify and separate by immunoprecipitation two major, discrete HDL particles, one of which contained apoA-I and apoA-II (LP-A-I:A-II) and the other only apoA-I (LP-A-I) as the integral protein constituents (4). This finding has been confirmed by several authors utilizing immunoaffinity chromatography with polyclonal antisera to apoA-I and apoA-II as the procedure of choice for isolating LP-A-I and LP-A-I:A-II (5–10). The present study shows that immunosorbents with pan-MAb to apoA-I and apoA-II may also be used for the fractionation and quantitative isolation of these two lipoprotein families in agreement with similar results recently reported by James *et al.* (11). It should be pointed out that MAb to apoA-I used in both of these studies are directed to the C-terminal region of apoA-I shown to be well expressed on the surface of lipoprotein particles (38, 39).

It has been shown in this comparative study that sexually mature males and females had higher concentrations of all lipids and apolipoproteins than prepubertal children. There were no differences in the lipid levels and only slight differences in the

Table 7. Plasma concentrations of minor apolipoproteins associated with LP-A-I and LP-A-I:A-II particles*

Subjects	Apolipoproteins (mg/dL)							
	C-II		C-III		D		E	
	LP-A-I	LP-A-I:A-II	LP-A-I	LP-A-I:A-II	LP-A-I	LP-A-I:A-II	LP-A-I	LP-A-I:A-II
Children (n = 16)	0.43 ^a (0.22)	1.32 (0.60)	2.11 ^b (1.19)	2.25 ^c (1.17)	1.82 (0.71)	5.85 ^{d,e} (1.39)	1.68 (1.41)	6.74 (2.28)
Adult males (n = 7)	0.21 ^a (0.25)	1.51 (0.87)	1.68 (2.39)	3.06 (2.39)	1.92 (1.00)	10.87 ^d (1.55)	1.78 (2.38)	4.53 ^f (1.23)
Adult females (n = 8)	0.48 (0.40)	1.79 (0.74)	1.12 ^b (0.80)	4.66 ^c (2.05)	2.53 (2.22)	8.42 ^e (2.81)	0.78 (1.25)	8.15 ^f (3.18)

* Values are mean ± (SD). Means marked with the same letter are significantly different: ^{a,b,f} $p < 0.05$; ^{c,e} $p < 0.01$; ^d $p < 0.001$.

Table 8. Percent lipid and apolipoprotein composition of LP-A-I and LP-A-I:A-II particles*

Subjects	Lipids	Apolipoproteins	Lipid/apolipoprotein ratio
LP-A-I			
Children (n = 16)	62.9 ± 5.1	37.1 ± 5.1	1.69
Adult males (n = 7)	50.1 ± 2.5†	49.9 ± 2.5	1.00
Adult females (n = 8)	61.0 ± 2.3	39.0 ± 2.3	1.56
LP-A-I:A-II			
Children (n = 16)	50.8 ± 2.9	49.2 ± 2.9	1.03
Adult males (n = 7)	44.2 ± 2.9†	55.8 ± 2.9	0.79
Adult females (n = 8)	51.3 ± 3.9	48.7 ± 3.9	1.05

* Values are mean ± SD.

† Mean significantly different ($p < 0.001$) from those of adult females and children.

concentrations of apoC-II, -C-III, and -E between prepubertal boys and girls. However, the concentration and chemical composition of LP-A-I and LP-A-I:A-II isolated from the prepubertal children differed from those isolated from men and women. These results also provided evidence that the already documented sex-related difference in the concentration and composition of these two lipoprotein families in the adult population (10, 40) occurs after sexual maturation.

The percentage distribution of apoA-I between LP-A-I and LP-A-I:A-II in children was similar to that in adults, indicating that 25–40% of the total apoA-I remains in LP-A-I. Although within a similar range, these results tend to be slightly lower than the reported values for the percentage of apoA-I (30–45%) associated with LP-A-I (8–11, 40–42). However, in contrast to a similar distribution of apoA-I between LP-A-I and LP-A-I:A-II in all age groups of normolipidemic subjects, the concentrations of these lipoprotein families, expressed in terms of apoA-I contents, were found to be slightly lower in prepubertal children than in adults. The most characteristic change from prepubertal to adult state was a significant increase in the levels of LP-A-I in women with little or no change in men. Because there was no significant sex-related difference in concentrations of adult LP-A-I:A-II, it appears that the well-documented increase in the levels of apoA-I in women (2, 40, 43–45) is due mainly to the elevation of LP-A-I. The elevation of LP-A-I levels in women was also accompanied by a compositional change in this lipoprotein family in that the relative content of apoA-I increased and that of minor apolipoproteins decreased in comparison with its childhood form. These results confirm and extend those of Koren *et al.* (40) and März *et al.* (46), who have also concluded that the higher concentrations of plasma apoA-I observed in women than in men may be accounted for by the higher concentration of LP-A-I. Ohta *et al.* (42) have also reported that women have higher levels of LP-A-I than men but, in contrast to findings of this study, they have ascribed this difference to a decrease of LP-A-I in men rather than an increase of these particles in women. The reason(s) for this discrepancy is not obvious but may possibly be due to the selection of adult populations in these two studies. In the former study, both men and women were younger than in the present study and had similar, if not identical, levels of plasma apoA-I. In view of the slightly decreasing effect of testosterone (47) and increasing effect of estrogens (48) on the concentrations of plasma apoA-I, it is possible that even slight differences or changes in the ratios of these gonadal hormones may be an important factor in regulating the levels of LP-A-I.

It has recently been shown (41) that the concentration of LP-A-I, but not LP-A-I:A-II, is lower in male patients with angiographically documented coronary artery disease than in patients without the disease or in normal controls. This observation may be accounted for by the capacity of LP-A-I, but not LP-A-I:A-II, to acquire cholesterol from cultured peripheral cells (49, 50), suggesting that the potentially antiatherogenic effect of LP-A-I might be mediated through their participation in reverse cholesterol transport.

Although the concentration of apoA-I in LP-A-I:A-II was

slightly higher in adult women than in children or adult males, these differences were not statistically significant. Age or sex had no effect on the percent lipid composition of LP-A-I:A-II, but some significant changes occurred in the apolipoprotein composition of this lipoprotein family. Among these, the most significant was that LP-A-I:A-II from men had lower apoA-I and apoE and higher apoD contents than those from children and women. In contrast to some experimental data supporting the putative antiatherogenic potential of LP-A-I, no such evidence is available for a similar antiatherogenic effect of LP-A-I:A-II. However, LP-A-I:A-II might exert such effect in their role as a possible link in the well-documented inverse relationship between HDL and TG-rich lipoproteins (51). It has been proposed that one of the main functions of LP-A-I:A-II (52) may be to serve as the major acceptor and reservoir of apoC-peptides and apoE released during the lipolytic degradation of TG-rich lipoproteins (35, 52, 53). Results of this study have now demonstrated that, regardless of sex and age, LP-A-I:A-II contain 70–90% of the total HDL content of apoC-II, -C-III, -D, and -E and, thus, may function as the main acceptors of these apolipoproteins (Table 7). A similar distribution of apoD and apoE was also reported by Cheung and Albers (54). Minor sex-related differences include a higher proportion of apoC-III in adult LP-A-I:A-II and a predominance of apoD in male and apoE in female LP-A-I:A-II.

This study has also revealed some interesting sex- and age-related differences in the chemical structure of LP-A-I and LP-A-I:A-II. A comparison of lipid composition between homologous LP-A-I and LP-A-I:A-II has shown that the CE/FC ratios of the former are always higher than those of the latter in agreement with similar findings by other investigators (10, 42, 54, 55). However, the lipid/apolipoprotein ratios of LP-A-I and LP-A-I:A-II in men were found to be significantly lower than in children or women, providing at least a partial explanation for the relatively low concentration of HDL₂ subfraction in men in comparison with women (34). Another significant sex-related structural difference pertains to the molar ratios of apoA-I/apoA-II in LP-A-I:A-II decreasing in the order of women (1.02) > children (0.90) > men (0.80). These values differ from those (1.5–2.0) reported in the literature (8–10). Because most results on apoA-I measurements in plasma are within a relatively narrow range of values, the observed difference in the apoA-I/apoA-II ratio is most probably due to the use of different secondary standards for determining the absolute concentration of apoA-II. However, whatever the true value of apoA-II may be, the difference in the observed molar ratios of apoA-I/apoA-II seems to reflect a sex- and age-related heterogeneity of LP-A-I:A-II. Before discussing the possible causes of this heterogeneity, it should be pointed out that apoA-I accounts for approximately 85% of the total protein of LP-A-I, whereas apoA-I and apoA-II account for approximately the same percentage of the total protein of LP-A-I:A-II. Because they occur in nonmolar ratios, minor apolipoproteins cannot be present on all LP-A-I and LP-A-I:A-II. Preliminary results from this laboratory (unpublished experiments), based on sequential immunoprecipitation of these

two lipoprotein families with antisera to apoC-III, -D, -E, have shown that approximately 70–75% of LP-A-I and LP-A-I:A-II are devoid of all minor apolipoproteins and that apoD and apoE are apparently not bound to the same LP-A-I or LP-A-I:A-II. These results, consistent with the existence of complex subspecies such as LP-A-I:D:lecithin cholesterol acyltransferase:cholesterol ester transfer protein described recently by Francone *et al.* (56), demonstrate the microheterogeneity of LP-A-I and LP-A-I:A-II brought about and defined by varying contents and proportions of minor apolipoproteins. We speculated that this compositional heterogeneity of LP-A-I:A-II may be the underlying cause for the sex-related difference in the apoA-I/apoA-II molar ratio of this lipoprotein family. For example, the apoA-I/apoA-II molar ratio of a LP-A-I:A-II:D subfamily occurring mainly in men and that of a LP-A-I:A-II:E subfamily present in a higher concentration in women cannot be the same. The reason for this is the presence in LP-A-I:A-II:E of small amounts of the apoE:apoA-II complex (57); the presence of apoA-II in this complex as a monomer leads to an increased molar ratio of apoA-I/apoA-II.

Results of this study show that the age- and sex-related differences in the concentration and composition of apoA-containing lipoprotein families reflect changes in the protein moieties of these lipoproteins. Furthermore, LP-A-I:A-II may function as the main acceptor of apoC peptides and apoE released during the lipolytic degradation of TG-rich lipoproteins and both LP-A-I and LP-A-I:A-II may consist of several subfamilies differentiated on the basis of their apolipoprotein composition. Finally, these results support the already documented chemical and metabolic uniqueness of LP-A-I and LP-A-I:A-II (9, 34, 49, 50) and indicate the need for further studies on their composition and function.

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