Apolipoprotein A-I_{Milano}: sex-related differences in the concentration and composition of apoA-I- and apoB-containing lipoprotein particles

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Abstract The presence of apolipoprotein (apo) A-I_{Milano} (A-I_M) mutant of apoA-I has a marked effect on plasma lipoproteins of A-I_M carriers including variable hypertriglyceridemia, increased levels of very low density lipoproteins (VLDL), slightly elevated levels of triglyceride-enriched low density lipoproteins (LDL) and greatly reduced levels of high density lipoproteins (HDL). To gain further insight into this dyslipoproteinemic syndrome characterized clinically by the absence of coronary artery disease, we have determined the concentration and composition of apoA- and apoB-containing lipoprotein families in four male and four female carriers and corresponding normal controls. Results have shown that A-I_M carriers have significantly reduced levels of lipoprotein (LP) A-I (45%), LP-A-I:A-II (60%), and LP-A-II (70%) and significantly increased levels of cholesterol-rich LP-B (67%) and triglyceride-rich LP-B:C, LP-B:C:E, and LP-A-II:B:C:D:E (65%) particles compared to controls. However, there were significant sex-related differences in the levels of apoA-and apoB-containing lipoproteins. Female carriers had significantly higher concentrations of LP-A-I (39 ± 10 vs. 12 ± 6 mg/dl) and LP-A-I:A-II (48 ± 11 vs. 30 ± 6 mg/dl) than male carriers. Furthermore, female carriers had higher levels of LP-B:C (23 ± 18 vs. 6 ± 5 mg/dl) and LP-A-II:B:C:D:E (13 ± 6 vs. 2.3 ± 0.8 mg/dl) but lower concentrations of LP-B (103 ± 52 vs. 152 ± 54 mg/dl) and LP-B:C:E (5 ± 2.5 vs. 13 ± 8 mg/dl) than male carriers. In general, the levels of LP-A-I and LP-A-I:A-II particles correlated positively with the levels of all three types of triglyceride-rich lipoproteins (LP-B_c) and negatively with the levels of LP-B particles. A comparative study of lipoprotein families in several dyslipoproteinemic states characterized by low levels of HDL has indicated that the characteristic lipoprotein particle profile of A-I_M carriers results most probably from the selective effect of apoA-I_M mutant rather than a general reduction in HDL levels. It appears that increased levels of LP-A-II:B:C:D:E particles, an inefficient substrate for lipoprotein lipase, and structurally defective LP-A-I:A-II particles, the normal acceptors of minor apolipoproteins released during lipolysis of triglyceride-rich lipoproteins, may be the main contributing factors to moderate hypertriglyceridemia characteristic of A-I_M carriers.—**Bekaert, E. D., P. Alaupovic, C. S. Knight-Gibson, G. Franceschini, and C. R. Sirtori.** Apolipoprotein A-I_{Milano}: sex-related differences in the concentration and composition of apoA-I- and apoB-containing lipoprotein particles. *J. Lipid Res.* 1993. **34:** 111–123.

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The apolipoprotein (apo) A-I_{Milano} (A-I_M) variant is characterized by a cysteine for arginine substitution at the position 173 in the primary sequence of apoA-I (1–4). As a consequence of this mutation, apoA-I_M variant forms disulfide linked homodimers (apoA-I_M/apoA-I_M) and heterodimers with apoA-II (apoA-I_M/apoA-II). Its presence has a significant effect on the structure and composition of plasma lipoproteins in general and high density lipoproteins (HDL) in particular (1, 5–8). ApoA-I_M carriers are characterized by variable hypertriglyceridemia accompanied by slightly increased concentrations of very low density lipoproteins (VLDL), normal or slightly increased levels of triglyceride-enriched low density lipoproteins (LDL), and greatly reduced concentrations of HDL particles (1, 6). Significantly decreased levels of apolipoproteins A-I and A-II coincide with a markedly reduced HDL_2 subfraction (30–35% of the normal concentration) and a lowered HDL_3 subfraction (60% of the normal concentration) (7). When compared to normal HDL_3, structurally and compositionally altered HDL_3 from apoA-I_M carriers occur as particles of three distinct sizes characterized by increased percentages of protein and triglyceride and a decreased percentage of cholesteryl esters. These changes have been attributed to the presence of apoA-I_M mutant and its homo- and heterodimers (7,

Abbreviations: apo, apolipoprotein; LP, lipoprotein; A-I_M, apolipoprotein A-I_{Milano} variant; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; EDTA, ethylenediamine tetraacetate; HS, heparin-Mn^{2+} supernate; HP, heparin-Mn^{2+} precipitate.

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8). This observation has been substantiated by results of turnover studies demonstrating that apoA-I_M dimer is catabolized at a slower rate than the normal apoA-I (9). In contrast, the apoA-I_M monomer has been shown to interact with increased flexibility with lipids (10) and to be catabolized at a faster rate than the normal apoA-I (9). It has been suggested that the severity of altered lipoprotein system in apoA-I_M carriers may depend on the proportions of normal apoA-I, hyperfunctional apoA-I_M monomer, and functionally defective apoA-I_M dimer (11). The clinically intriguing aspect of this dyslipoproteinemic state is that, in spite of an unfavorable lipoprotein profile, apoA-I_M carriers have very low, if any, incidence of coronary artery disease (1, 11).

To further characterize plasma lipoproteins in this intriguing dyslipoproteinemic syndrome, we have studied the concentration and composition of apoA- and apoB-containing lipoprotein families identified and defined by their apolipoprotein composition. It has already been established that HDL of apoA-I_M carriers consist of lipoprotein A-I (LP-A-I) and lipoprotein A-I:A-II (LP-A-I:A-II) particles present in reduced but similar proportions as in normal subjects (12). The present paper, based on a larger number of apoA-I_M carriers, confirms and extends these findings by demonstrating sex-related differences in the concentration of major apoA-containing lipoprotein families and by providing evidence for the presence of not only LP-A-I and LP-A-I:A-II particles but also lipoprotein A-II (LP-A-II) particles (13) of distinct lipid and apolipoprotein composition. Because the presence of apoA-I_M also affects the VLDL and LDL, it was considered important to fractionate this group of lipoproteins into four major apoB-containing lipoprotein families (14) and to determine their concentrations in female and male A-I_M carriers. The present results show that A-I_M carriers have increased concentrations of all four major apoB-containing lipoprotein families in comparison with normal controls. Moreover, the sex-related difference in the concentration and composition of apoA-containing lipoproteins also applies to apoB-containing lipoproteins in that male carriers have higher levels of cholesteryl ester-rich lipoprotein B (LP-B) and triglyceride-rich lipoprotein B:C-I:C-II:C-III:E (LP-B:C:E) and female carriers have higher levels of triglyceride-rich lipoprotein B:C-I:C-II:C-III (LP-B:C) and lipoprotein A-II:B:C-I:C-II:C-III:D:E (LP-A-II:B:C:D:E or LP-A-II:B complex).

MATERIALS AND METHODS

Subjects

This study was conducted with four female (pedigree identification VI-176, VII-149, VII-201, and VII-206) and four male (pedigree identification VI-171, VI-207, VII-46, and VII-134) apoA-I_M carriers (mean age 34 ± 9 years) whose biochemical and clinical characteristics have been previously described (15). Because it was not possible to obtain blood samples from relatives of A-I_M carriers as control subjects, we used recently described sex- (eight women and seven men) and age- (mean age 36 ± 9 years) matched asymptomatic, normolipidemic subjects as controls (16). There were no significant differences in the concentrations of plasma total cholesterol, HDL-cholesterol, apoA-I, and apoB between controls used in this study and previously described relatives of A-I_M carriers (6) except for slightly elevated but normal triglyceride levels (68 ± 41 vs. 126 ± 70 mg/dl, $P < 0.05$) in the latter subjects. Differences in the lipid and apolipoprotein concentrations between A-I_M carriers participating in this study and their previously described relatives (6) reached the same level of statistical significance as those between A-I_M carriers and present controls.

Patients with primary and secondary hyperlipoproteinemia selected for the measurement of apoA- and apoB-containing lipoprotein families were previously described, including patients with primary, nonfamilial hypercholesterolemia and clinically documented coronary artery disease (17), patients with chronic renal failure (18), and patients with glycogen storage disease, type I (19).

Venous blood was drawn into EDTA-containing Vacutainer tubes from normolipidemic and dyslipoproteinemic subjects after an overnight fast and the plasma samples were collected by low-speed centrifugation. Plasma samples from apoA-I_M carriers were immediately air-shipped on ice from Milan to Oklahoma City. Upon receipt, preservatives were added to final concentrations of 500 units/ml penicillin-G, 50 μg/ml streptomycin sulfate, 1.3 mg/ml ε-amino caproic acid, and 0.5 mg/ml reduced glutathione (20). The measurement of lipids and apolipoproteins commenced 48–96 h after blood collection. All blood donors gave informed consent for the study.

Preparation of immunosorbers

The preparation and characterization of "pan" monoclonal antibodies to apoB (21) and apoA-II (22) have been previously described. A monoclonal antibody to apoA-I (HB-22) was a gift from Ms. Teva C. Rothwell, Hycor Biomedical Inc., Fountain Valley, CA. This antibody had all the characteristics of a "pan" antibody, because when coupled to agarose activated with N-hydroxysuccinimide (Affi-Gel 10, Bio-Rad Laboratories, Richmond, CA) it retained all lipoprotein forms of apoA-I. The immunosorbers were prepared according to a previously reported procedure (23).

Isolation of apoA-I- and apoA-II-containing lipoproteins

Apolipoprotein A-I occurs as a protein constituent of two distinct lipoprotein families of particles, i.e., LP-A-I

and LP-A-I:A-II. These lipoprotein families were isolated from whole plasma by immunoaffinity chromatography on anti-apoA-I- and anti-apoA-II-containing immunosorbers and quantified according to a previously described procedure (16). The recoveries of apoA-I ranged between 80 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.

Due to the presence of apoA-II in three distinct lipoproteins including LP-A-II, LP-A-I:A-II, and LP-A-II:B complex, the fractionation of these lipoprotein families was performed by a recently described sequential, three-step immunoaffinity chromatography with anti-apoA-II, anti-apoB, and anti-apoA-I immunosorbers (24). The recoveries of apoA-II were between 76 and 92% of applied apoA-II. The plasma levels of apoA-II associated with LP-A-II particles were estimated on the basis of plasma apoA-II values and the percent distribution of apoA-II in retained and unretained fractions after chromatography on anti-apoB and anti-apoA-I immunosorbers.

Fractionation of apoB-containing lipoprotein particles

Fractionation of apoB-containing lipoproteins into cholesteryl ester-rich LP-B and triglyceride-rich LP-B:C, LP-B:C:E, and LP-A-II:B complex particles was performed according to a procedure (24) based on the separation of LP-A-II:B complex by immunoaffinity chromatography on an anti-apoA-II immunosorber (22) and fractionation of remaining apoB-containing lipoproteins by sequential immunoprecipitation with polyclonal antisera to apoE and apoC-III, respectively (25). This procedure was applied to apoB-containing lipoproteins separated from apoA-containing lipoproteins by affinity chromatography of whole plasma on concanavalin A (ConA) (26).

The retained fraction from the ConA column (apoB-containing lipoproteins) was characterized by quantitative estimation of apoB and the LP-A-II complex was separated from other apoB-containing lipoproteins by immunoaffinity chromatography on an anti-apoA-II immunosorber as previously described (22). The unretained fraction was concentrated to approximately one-third of its volume and used in the next step for the sequential immunoprecipitation with polyclonal antisera to apoE and apoC-III according to a previously described procedure (25, 27). Concentrations of all four apoB-containing lipoproteins (LP-B, LP-B:C, LP-B:C:E, and LP-A-II:B complex) were expressed in terms of apoB (mg/dl). The recoveries of apoB ranged between 70 and 95% of applied apoB; the levels of apoB in individual lipoprotein families were determined on the basis of plasma apoB values and the percent distribution of apoB in corresponding lipoproteins. Because plasma samples from normolipidemic control subjects were not fractionated on the anti-apoA-II immunosorber prior to their separation by sequential immunoprecipitation, the lipoproteins precipitated by anti-apoE serum consisted of both the LP-B:C:E and LP-A-II:B complex particles.

Determination of apoC-III distribution (apoC-III-ratio) in lipoproteins isolated by heparin-Mn^{2+} procedure

The ratio of apoC-III (apoC-III-ratio) in heparin-Mn^{2+} supernate (HS) and heparin-Mn^{2+} precipitate (HP) has been introduced as a means of assessing the efficiency of processes responsible for the degradation of triglyceride-rich lipoprotein particles (17, 28). The conceptual basis and validation of apoC-III-ratio has been previously described (28). The heparin-Mn^{2+} treatment of whole plasma (29) and the measurement of apoC-III (19) in heparin-Mn^{2+} supernates and precipitates were described by previously reported procedures.

Crossed immunoelectrophoresis

Immunochemical characterization of apoA-II-containing lipoprotein particles was performed by crossed immunoelectrophoresis according to a previously described procedure (30).

Lipid, apolipoprotein, and lipoprotein analyses

Plasma total cholesterol and triglycerides were determined as previously described (16). The contents of cholesteryl esters, free cholesterol, and triglycerides in isolated lipoprotein particles were determined by the gas–liquid chromatographic method of Kuksis et al. (31). The phospholipid phosphorus content was measured by the procedure of Gerlach and Deuticke (32). The VLDL, LDL, and HDL were isolated by sequential preparative ultracentrifugation at solution densities 1.006 g/ml, 1.063 g/ml, and 1.21 g/ml, respectively, as previously reported (33). Apolipoproteins A-I, A-II, B, C-II, C-III, D, and E were quantified by electroimmunoassays developed in this laboratory (16, 22, 23).

Statistical methods

Means and SD were calculated according to standard methods. Significance of differences was estimated by Wilcoxon rank sum test. Pearson's correlation coefficient, r, was used to estimate the degree of association between two variables. The level of significance was taken as $P < 0.05$.

RESULTS

Plasma lipids and apolipoproteins

Male and female apoA-I_M carriers had significantly higher concentrations of plasma triglycerides, VLDL-

TABLE 1. Concentrations of lipids and apolipoproteins in whole plasma of A-I_{Milano} carriers and normal controls

Lipids and Apolipoproteins	A-I Milano Carriers			Controls
	4F + 4M[a]	4F	4M	8F/7M
		mg/dl		*mg/dl*
Total cholesterol	187 ± 59[b]	181 ± 60	193 ± 68	213 ± 37
Triglycerides	203 ± 117[b]	206 ± 144	201 ± 105	68 ± 41
VLDL-cholesterol	49 ± 23[b]	52 ± 23	45 ± 26	17 ± 9
LDL-cholesterol	134 ± 34	126 ± 17	146 ± 51	106 ± 29
HDL-cholesterol	19 ± 6[b]	24 ± 4[d]	14 ± 2	59 ± 10
ApoA-I	64 ± 27[c]	85 ± 20[d]	42 ± 12	143 ± 17
ApoA-II	37 ± 8[b]	44 ± 3[d]	31 ± 6	80 ± 10
ApoB	154 ± 53[b]	140 ± 56	173 ± 53	92 ± 23
ApoC-II	3.2 ± 1.3	3.6 ± 1.3	2.6 ± 1.5	2.7 ± 1.1
ApoC-III	8.9 ± 3.9	9.9 ± 4.1	7.6 ± 3.8	9.1 ± 2.4
ApoD	9.7 ± 2.1[a]	10.2 ± 2.2	9.2 ± 2.2	15.2 ± 3.7
ApoE	9.6 ± 3.2	10.2 ± 4.0	8.7 ± 2.2	11.5 ± 3.9

Values given as mean ± SD. F, female; M, male.
[a] $P < 0.01$; [b], $P < 0.001$; [c], $P < 0.0001$: significance of difference between A-I_{Milano} carriers and normal controls.
[d], $P < 0.01$: significance of difference between A-I_{Milano} female and male carriers.

cholesterol, and apoB and significantly lower concentrations of HDL-cholesterol, apoA-I, apoA-II, and apoD than sex- and age-matched normolipidemic controls (**Table 1**). There were no significant differences in the levels of total cholesterol and LDL-cholesterol, although the levels of former tended to be slightly higher in controls and those of latter in apoA-I_M carriers: the increased levels of apoB in apoA-I_M carriers were not accompanied by corresponding increases in apolipoproteins C-II, C-III, and E as already observed by Franceschini et al. (6).

Female A-I_M carriers had significantly higher concentrations of plasma HDL-cholesterol and apolipoproteins A-I and A-II than male A-I_M carriers (Table 1), but there was no difference in the levels of total cholesterol, triglycerides, and apoD. The female carriers tended to have lower levels of apoB and slightly higher levels of apoC-polypeptides and apoE than male carriers commensurate with their lower concentrations of LDL-cholesterol and higher concentrations of VLDL-cholesterol.

The apparent discrepancy in A-I_M carriers between relatively high plasma concentrations of apoB and normal levels of apoC-peptides and apoE seems to be due mainly to reduced concentrations of these latter apolipoproteins in HDL particles as indicated by significantly decreased levels of apoC-III-HS in comparison with normal controls (**Table 2**). The concentration of apoC-III-HS in male carriers was even lower than that in female carriers (Table 2). However, the concentrations of apoC-III-HP in both female and male carriers were significantly higher than in control subjects (Table 2). As a result of this abnormal distribution of apoC-III between heparin supernates ("HDL") and precipitates ("VLDL + LDL"), the A-I_M carriers had a significantly lower apoC-III-ratio than controls. This finding in conjunction with increased concentrations of plasma triglycerides, VLDL-cholesterol, and apoB suggested the occurrence in A-I_M carriers of increased concentrations of intact and/or partially degraded triglyceride-rich lipoproteins. It appeared that triglyceride-rich lipoproteins accumulated to a greater extent in female than in male carriers (Tables 1 and 2). The increased concentrations of apoB (15.8 ± 8.0 vs. 2.7 ± 0.2 mg/dl) and apoC-III (3.4 ± 2.0 vs. 0.4 ± 0.1 mg/dl) but similar neutral lipid/apoB and neutral lipid/apoC-III ratios in VLDL and LDL_1 ($d < 1.020$ g/ml) of A-I_M carriers indicated that elevated concentrations of triglyceride-rich lipoproteins were due to an increased number rather than lipid enrichment of these particles.

TABLE 2. Concentrations of apoC-III in heparin supernates and heparin precipitates of A-I_{Milano} carriers and normal controls

Subjects	Sex	ApoC-III-HS	ApoC-III-HP	ApoC-III-Ratio
		mg/d	*mg/dl*	
A-I_{Milano}	4F/3M	1.7 ± 0.8	6.6 ± 4.4	0.5 ± 0.5
Controls[a]	76F/63M	5.1 ± 1.7[b]	3.0 ± 1.4[b]	2.1 ± 1.0[b]
A-I_{Milano}	4F	2.3 ± 0.6	7.4 ± 5.3	0.6 ± 0.7
A-I_{Milano}	3M	1.0 ± 0.3[c]	5.6 ± 3.9	0.3 ± 0.2

Values given as mean ± SD. F, female; M, male.
[a] Control subjects include 139 (76F + 63M) normolipidemic, asymptomatic subjects randomly selected from among employees of the Oklahoma Medical Research Foundation.
[b], $P < 0.001$: significance of difference between A-I_{Milano} and normal controls.
[c], $P < 0.05$: significance of difference between A-I_{Milano} female and male carriers.

TABLE 3. Plasma levels of apoA-I and apoA-II associated with LP-A-I, LP-A-I:A-II, and LP-A-II of A-I_{Milano} carriers and normal controls

Subjects	Sex	Apolipoprotein A-I		Apolipoprotein A-II	
		LP-A-I	LP-A-I:A-II	LP-A-II	LP-A-I:A-II
		mg/dl (%)		*mg/dl (%)*	
A-I Milano	4F/4M	25.6 ± 16.1 (36.9 ± 9.8)	38.3 ± 12.1 (63.1 ± 9.8)	2.6 ± 1.2[a] (6.2 ± 3.2)	39.4 ± 4.0 (93.7 ± 3.2)
Normals	8F/7M	47.2 ± 1.34[d] (31.9 ± 8.3)	95.7 ± 6.4[d] (68.0 ± 6.4)	8.9 ± 4.9[b] (10.5 ± 5.5)	76.1 ± 10.8[d] (89.5 ± 5.5)
A-I Milano	4F	38.9 ± 9.8 (45.3 ± 2.9)	46.7 ± 10.7 (54.7 ± 2.9)	2.3 ± 1.3 (5.4 ± 3.2)	40.7 ± 3.8 (94.6 ± 3.2)
Normals	8F	54.4 ± 11.0[b] (35.6 ± 4.6)	97.3 ± 7.4[d] (64.4 ± 4.6)		
A-I Milano	4M	12.4 ± 5.7[f] (28.5 ± 5.2)[f]	29.9 ± 6.4[e] (71.1 ± 5.2)‡‡	3.5 (8.9)	35.7 (91.1)
Normals	7M	38.5 ± 11.1[c] (28.9 ± 6.2)	93 ± 9.3[d] (71.1 ± 6.2)		

Values given as mean ± SD; values in parentheses are relative distribution (%) of apoA-I or apoA-II. F, female; M, male.

[a] A-I_{Milano} carriers include 3F/1M; normal controls include 2F/4M.

[b], $P < 0.05$; [c], $P < 0.005$; [d], $P < 0.001$: significance of difference between A-I_{Milano} carriers and normal controls.

[e], $P < 0.01$; [f], $P < 0.005$: significance of difference between A-I_{Milano} female and male carriers.

Plasma concentrations of apoA-I associated with LP-A-I and LP-A-I:A-II particles

Although the percentage distribution of apoA-I between LP-A-I and LP-A-I:A-II particles in apoA-I_M carriers was similar to that in normal controls, the levels of apoA-I associated with these two lipoprotein families were significantly lower ($P < 0.001$) in carriers than controls (**Table 3**). The decrease in the levels of LP-A-I particles (46%) was slightly lower than the corresponding decrease in the levels of LP-A-I:A-II particles (60%). When compared to controls, both the female and male apoA-I_M carriers had significantly lower levels of LP-A-I and LP-A-I:A-II particles.

The difference in the levels of plasma apoA-I and apoA-II between female and male apoA-I_M carriers was also reflected in their levels of LP-A-I and LP-A-I:A-II particles. Male carriers had significantly lower concentrations of LP-A-I:A-II and, especially, LP-A-I than female carriers. Consequently, they also had a significantly lower percentage of apoA-I in LP-A-I particles and a higher percentage in LP-A-I:A-II particles (Table 3).

Plasma concentrations of apoA-II associated with LP-A-II and LP-A-I:A-II

We have recently isolated and characterized LP-A-II particles from normolipidemic subjects and patients with apoA-I and apoA-I/apoC-III deficiencies (13, 21) confirming the previously reported presence of this minor HDL family in normal (34) and dyslipoproteinemic (30, 35, 36) subjects. To establish the possible presence of LP-A-II particles in apoA-I_M carriers, their whole plasma was chromatographed on an anti-apoA-II immunosorber and the retained fraction consisting of all apoA-II-containing lipoproteins was tested by crossed immunoelectrophoresis (**Fig. 1**). Electrophoresis of apoA-II-containing lipoproteins through agarose layers with incorporated antisera to apoB, apoA-I, and apoA-II showed three distinct precipitin lines ("rockets") with the slow-moving LP-A-II:B complex particles in the bottom gel,

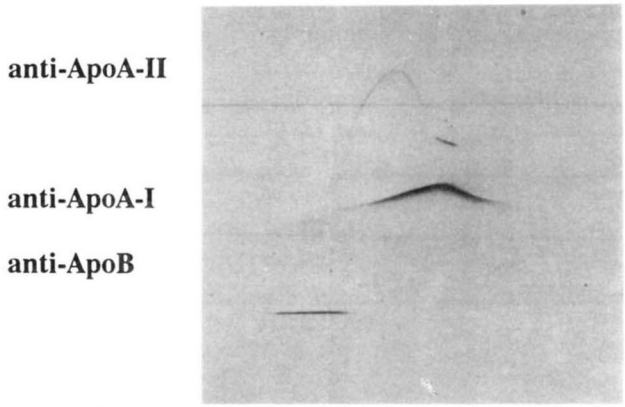

Fig. 1. Crossed immunoelectrophoresis of apoA-II-containing lipoproteins isolated from whole plasma of an apoA-I_M carrier (VI-207) on an anti-apoA-II immunosorber (the retained fraction). Anti-apoB serum is incorporated in the lower gel, anti-apoA-I serum in the intermediate gel, and anti-apoA-II serum in the upper gel. Experimental conditions are described in Methods.

LP-A-I:A-II particles in the intermediate gel, and LP-A-II particles in the upper gel. To confirm the occurrence in apoA-I_M carriers of LP-A-II particles, apoA-II-containing lipoproteins were separated into three fractions by sequential immunoaffinity chromatography and their concentrations were determined by measurement of apoA-II contents. After correcting for the apoA-II content of LP-A-II:B complex particles, the distribution of remaining apoA-II between the major LP-A-I:A-II and minor LP-A-II particles was used for determining their plasma concentrations. The homogeneity of LP-A-II-containing fraction was ascertained by the immunochemically and electrophoretically determined absence of apoA-I.

The percentage distribution of apoA-II between LP-A-II and LP-A-I:A-II particles was similar in apoA-I_M carriers and normal controls (Table 3). However, the concentrations of both LP-A-II and LP-A-I:A-II, expressed in terms of apoA-II contents, were significantly lower in apoA-I_M carriers than in normal subjects.

Lipid and apolipoprotein composition of apoA-containing lipoproteins

The lipid/protein ratios of LP-A-I, LP-A-I:A-II, and LP-A-II particles from A-I_M carriers taken as a group were slightly higher than those of corresponding normal controls (1.6 vs. 1.2, 1.1 vs. 0.9, and 3.5 vs. 2.1, respectively). However, the lipid/protein ratios of LP-A-I and LP-A-I:A-II particles from male A-I_M carriers were higher than those from female carriers (2.7 vs. 1.1 and 1.3 vs. 0.9, respectively). In controls, these ratios were reversed in that men had lower lipid/protein ratios of LP-A-I and LP-A-I:A-II particles than women (1.0 vs. 1.6 and 0.8 vs. 1.1, respectively).

The lipid compositions of LP-A-I and LP-A-I:A-II particles from A-I_M carriers were characterized by significantly higher percentages of triglycerides (7.6 ± 4.1 vs. 2.8 ± 1.6%, $P < 0.001$; and 9.0 ± 3.3 vs. 3.4 ± 1.5, $P < 0.001$, respectively) and lower percentages of cholesteryl esters (13.6 ± 4.8 vs. 24.2 ± 6.7%, $P < 0.001$; and 17.4 ± 4.4 vs. 32.3 ± 4.5%, $P < 0.005$, respectively) than those of corresponding lipoprotein particles from control subjects. LP-A-II particles from A-I_M carriers had a significantly lower percentage of cholesteryl esters (5.2 ± 2.6 vs. 12.3 ± 3.9%, $P < 0.005$) than those from normal subjects. There were no significant differences in the relative contents of free cholesterol and phospholipids with the exception of LP-A-I:A-II particles which, in A-I_M carriers, had significantly lower percentage of free cholesterol (4.9 ± 0.9 vs. 5.9 ± 1.1%, $P < 0.05$) and higher percentage of phospholipids (68.5 ± 5.8 vs. 58.2 ± 5.2%, $P < 0.001$) than in control subjects. As a result of these differences, all three apoA-containing lipoprotein families from A-I_M carriers had significantly lower cholesteryl ester/triglyceride ratios than those from normal controls. Males tended to have slightly higher percentages of triglycerides and lower percentages of cholesteryl esters than female carriers.

In LP-A-I and LP-A-I:A-II particles from A-I_M carriers and normal controls the protein moieties consisted of approximately 86–91% apoA-I or apoA-I and apoA-II, and 9–14% minor apolipoproteins (**Table 4**). When com-

TABLE 4. Apolipoprotein composition of LP-A-I and LP-A-I:A-II from A-I_{Milano} carriers and normal controls

Subjects	Sex	Apolipoproteins					
		A-I	A-II	C-II	C-III	D	E
		%					
LP-A-I							
A-I_{Milano}	4F/4M	86 ± 8[a]	0[b]	0.5 ± 0.6	2.2 ± 2.5	2.3 ± 2.5	8.5 ± 8.9
Controls	8F/6M	89 ± 7	0	0.6 ± 0.6	2.3 ± 2.5	5.7 ± 3.4[c]	2.3 ± 2.5[c]
LP-A-I:A-II							
A-I_{Milano}	4F/4M	52 ± 4	39 ± 4	0.5 ± 0.4	1.1 ± 1.0	3.8 ± 1.0	3.8 ± 1.8
Controls	8F/6M	51 ± 4	35 ± 5	0.9 ± 0.4[c]	2.4 ± 1.7	6.2 ± 1.7[d]	4.2 ± 2.8
LP-A-II							
A-I_{Milano}	VII-201/F	0	41.3	0	0	0	56.9
	VII-206/F	0	89.4	0	0	10.5	0
	VI-176/F	0	42.8	0	21.4	10.5	25.0
Controls	2M/1F	0	77.4 ± 36	0	0	22.6 ± 3.6	0
	1M/1F	0	100.0	0	0	0	0

[a] Mean ± SD.
[b] Sign "0" indicates not detectable by electroimmunoassay.
[c], $P < 0.05$; [d], $P < 0.005$: significance of difference between A-I_{Milano} carriers and normal controls.

pared to normal controls, the LP-A-I particles from A-I_M carriers had a significantly lower percentage of apoD and a higher percentage of apoE. The apolipoprotein composition of LP-A-I:A-II particles from apoA-I_M carriers was similar to that of LP-A-I:A-II particles from control subjects except for a significantly lower percentage of apoC-II and apoD. There were no significant differences in the apolipoprotein composition of these two major lipoprotein families between female and male A-I_M carriers. Our measurements suggest that, in LP-A-I:A-II particles from female A-I_M carriers and normal controls, the apoA-I/apoA-II molar ratio was close to unity; in male A-I_M carriers the apoA-I/apoA-II molar ratio was 0.75 suggesting a difference in the composition of apoA-I_M monomers and heterodimers between male and female carriers.

The protein moiety of LP-A-II particles in normal controls consisted in most cases studied either of apoA-II and apoD or of apoA-II as the sole apolipoprotein constituent (Table 4). In contrast, the apolipoprotein composition of LP-A-II particles from A-I_M carriers was characterized not only by the presence of apoD as one of its minor apolipoproteins but also by relatively high percentages of apoC-III and/or apoE (Table 4). In this respect, LP-A-II particles from apoA-I_M carriers resembled LP-A-II particles from patients with apoA-I deficiency syndromes (24) where they seem to function in the absence of LP-A-I and LP-A-I:A-II particles as substitute acceptors for apoC-peptides and apoE.

Distribution of minor apolipoproteins among apoA-I-containing lipoprotein particles

There was no significant difference between A-I_M carriers and normal controls in the total concentrations of minor apolipoproteins associated with apoA-I-containing lipoproteins. However, in normolipidemic controls a slightly greater proportion (56%) of the total minor apolipoprotein content was associated with LP-A-I:A-II particles, while in A-I_M carriers a greater proportion (60%) of these apolipoproteins was associated with LP-A-I particles. In LP-A-I particles, apoD levels were significantly lower but apoE levels were significantly higher in A-I_M carriers than in control subjects (Table 4). In contrast, the levels of all minor apolipoproteins were lower in LP-A-I:A-II of A-I_M carriers than in normal controls (Table 4). Consequently, the total concentration of minor apolipoproteins associated with apoA-I-containing lipoproteins (mg/100 mg apoA-I) was slightly but insignificantly higher in LP-A-I (16.5 ± 10.7 vs. 12.9 ± 8.8, NS) and significantly lower in LP-A-I:A-II (17.8 ± 3.6 vs. 2.6 ± 9.2, $P < 0.05$) of A-I_M carriers than normal controls. These results have suggested that the presence of apoA-I mutant may have some effect on the association of minor apolipoproteins with apoA-containing lipoprotein particles.

Concentrations of apoB-containing lipoprotein particles

Although there was a marked variability among A-I_M carriers in the apoB levels, the great majority (7/9 or 78%) had apoB levels higher than 1 SD of the normal controls. The measurement of apoB-containing lipoprotein particles by a combination of immunoaffinity chromatography and sequential immunoprecipitation showed that A-I_M carriers had significantly higher concentrations of both cholesteryl ester-rich LP-B and triglyceride-rich LP-B_c particles (the sum of all triglyceride-rich lipoproteins) than normolipidemic controls (**Table 5**); however, there was no difference between A-I_M carriers and controls in the percentage distribution of these two types of apoB-containing lipoproteins. The estimation of three major triglyceride-rich lipoprotein families revealed that the levels of LP-B:C and apoE-containing LP-B:C:E and LP-A-II:B:C:D:E (LP-A-II:B complex) particles were higher in A-I_M carriers than in normal controls.

There were some marked sex-related differences in the levels of apoB-containing lipoproteins among A-I_M carriers. In general, female carriers had lower concentrations of cholesteryl ester-rich LP-B and higher concentrations

TABLE 5. Concentrations of apoB-containing lipoprotein particles in whole plasma of A-I_{Milano} carriers and normal controls

Subjects	Sex	LP-B	LP-B:C	LP-B:C:E	LP-A-II:B	LP-B:C:E + LP-A-II:B	LP-B_c
				mg/dl			
A-I_{Milano}	4F/3M	124 ± 54	16.0 ± 16.0	8.2 ± 6.6	8.4 ± 7.1	16.6 ± 5.5	32.4 ± 16.3
Controls	8F/7M	74 ± 19[b]	6.6 ± 5.4			12.8 ± 8.7	19.6 ± 11.2[a]
A-I_{Milano}	4F	103 ± 52	23 ± 18	4.6 ± 2.5	12.9 ± 6.1	17.5 ± 4.3	40.6 ± 15
A-I_{Milano}	3M	152 ± 54	6.1 ± 4.8	13.0 ± 7.9	2.3 ± 0.8[c]	15.3 ± 7.7	21.4 ± 12.1

Values are given as mean ± SD. F, female; M, male.
[a], $P < 0.05$; [b], $P < 0.01$: significance of difference between A-I_{Milano} and normal controls.
[c], $P < 0.05$: significance of difference between A-I_{Milano} female and male carriers.

of triglyceride-rich LP-B:C and LP-A-II:B complex particles than male carriers with the possible exception of LP-B:C:E particles (Table 5). However, due to some overlap and a relatively small number of subjects, these differences only reached a level of statistical significance in the case of LP-A-II:B complex particles. Thus, the male and female A-I_M carriers differed both in the apoA- and apoB-containing lipoprotein profiles. To further explore the relationship between the apoA- and apoB-containing lipoproteins, A-I_M carriers were divided into two subpopulations based on the cut-off point of apoA-I = 64 mg/dl, the mean value for apoA-I of the entire population. This subdivision mimicked very closely the already established sex-related differences in the levels of lipids and apolipoproteins. Carriers with relatively high levels of apoA-I (95 ± 12 mg/dl) had significantly higher levels of LP-A-I and LP-A-I:A-II particles and significantly lower concentrations of LP-B particles than carriers with low levels of apoA-I (46 ± 13 mg/dl) (**Table 6**); in contrast, the former carriers tended to have higher levels of LP-B:C and LP-A-II:B complex particles than the latter carriers. In A-I_M carriers as a group, the levels of LP-A-I and LP-A-I:A-II particles correlated negatively with the levels of LP-B particles ($r = -0.68$, respectively) but positively with the levels of LP-B_c particles ($r = 0.77$ and $r = 0.68$, respectively).

A comparison of apoA- and apoB-containing lipoprotein families between patients with low concentrations of HDL-cholesterol

To establish whether the profound changes in the concentration and composition of apoA- and apoB-containing lipoprotein families in A-I_M carriers may be ascribed specifically to the presence of apoA-I_M mutant or to generally low HDL-cholesterol values, we determined the levels of these lipoproteins in patients with some primary or secondary hyperlipoproteinemias selected on the basis of their low HDL-cholesterol concentrations (less than 32 mg/dl, range 20–32 mg/dl). As shown in **Table 7,** there were no differences in the concentrations of LP-A-I particles between A-I_M carriers taken as a group and patients with either primary nonfamilial hypercholesterolemia or secondary hypertriglyceridemia represented by patients with chronic renal failure or glycogen storage disease, type I; it is worthwhile noticing that female carriers tended to have a slightly higher average concentration of LP-A-I than hypercholesterolemic or hypertriglyceridemic patients, while the male carriers had some of the lowest recorded levels of this lipoprotein family. However, the most characteristic difference between A-I_M carriers and other dyslipoproteinemic patients was in the concentration of LP-A-I:A-II particles which were significantly lower ($P < 0.001$) in the former than in the latter subjects including those with high concentrations of triglycerides (Table 7). Another interesting difference, due most probably to the presence of apoA-I_M mutant, was in the relationship between apoA- and apoB-containing lipoproteins. As mentioned earlier, in A-I_M carriers both the LP-A-I and LP-A-I:A-II particles were related positively to LP-B_c and negatively to LP-B particles; in other dyslipoproteinemias, however, the relationship of LP-A-I particles to LP-B and LP-B_c was always opposite to that of LP-A-I:A-II particles. For example, in chronic renal failure, the LP-A-I particles were correlated negatively and the LP-A-I:A-II particles positively with LP-B and LP-B_c particles. Findings of this comparative study suggest that in A-I_M carriers the characteristic concentration profiles and relationship between apoA- and apoB-containing lipoprotein families result from the presence and specific effect of apoA-I_M mutant rather than the generally low levels of HDL.

DISCUSSION

Among the known apoA-I variants, apoA-I_M mutant appears to have the most profound effect on the composition and concentration of all major lipoprotein density classes (1, 4–10). The main goal of this study was to extend these findings by identifying and quantifying major apoA- and apoB-containing lipoprotein families, exploring their possible relationship and speculating about their potential clinical significance. Results of this study have confirmed that A-I_M carriers have significantly higher levels of triglycerides and VLDL-cholesterol, slightly increased

TABLE 6. ApoA- and apoB-containing lipoproteins in A-I_{Milano} carriers: effect of apoA-I concentration

Carriers	Sex	ApoA-I	ApoB	LP-A-I	LP-A-I:A-II	LP-B	LP-B:C	LP-B:C:E	LP-A-II:B
					mg/dl				
A-I_{Milano} ApoA-I > 64 mg/dl	4F	95 ± 12	112 ± 49	43 ± 6	50 ± 9	79 ± 26	27 ± 19	3.9 ± 2.5	13.5 ± 7.4
A-I_{Milano} ApoA-I ≤ 64 mg/dl	4M/1F	46 ± 13[c]	167 ± 49	15 ± 8[c]	31 ± 6[b]	157 ± 46[a]	7.1 ± 4.4	11.4 ± 7.2	4.5 ± 4.4

Values are given as mean ± SD. F, female; M, male.
[a], $P < 0.05$; [b], $P < 0.01$; [c], $P < 0.001$: significance of difference between A-I_{Milano} carriers with apoA-I levels below and above 64 mg/dl.

TABLE 7. Concentrations of apoA- and apoB-containing lipoprotein families of A-I_{Milano} carriers and patients with other dyslipoproteinemias

Subjects	TG	HDL-C	LP-A-I	LP-A-I:A-II	LP-B	LP-B_c
			mg/dl			
A-I_{Milano} (n = 8)	203 (117)	18.8 (5.7)	25.6 (16.1)	38.3 (12.1)	124 (54)	32.4 (16.3)
A-I_{Milano} Females (n = 4)	206 (144)	23.5 (4.2)	38.9 (9.8)	46.7 (10.7)	103 (52)	40.6 (15.0)
A-I_{Milano} Males (n = 4)	201 (110)	14.2 (1.5)	12.4 (5.7)	29.9 (6.4)	152 (54)	21.4 (12.1)
Primary hypercholesterolemia (n = 6)	139 (40)	27.0 (3.9)	37.1 (8.7)	65.9[d] (11.1)	96.0 (21.2)	25.7 (8.2)
Chronic renal failure (n = 9)	183 (77)	27.2 (4.6)	25.6 (9.0)	72.0[d] (15.1)	106 (16.7)	12.0[c] (9.5)
Glycogen storage disease-type I (n = 7)	618 (384)	27.7 (4.5)	26.2[a]	71.7[a]	93.6 (32.2)	94.3[b] (57.3)
Normal controls (n = 7)	69.4 (24.5)	49.7 (8.0)	47.8[b] (18.1)	89.0[d] (22.7)	81.6 (28.9)	11.3[c] (8.8)

Values are given as mean ± SD.
[a] Measurement of LP-A-I and LP-A-I:A-II was performed on two patients.
[b], $P < 0.05$; [c], $P < 0.01$; [d], $P < 0.001$: significance of difference between A-I_{Milano} female and male carriers and normolipidemic and hyperlipoproteinemic subjects.

concentrations of LDL-cholesterol, and significantly lower levels of plasma cholesterol esters, HDL-cholesterol, and apolipoproteins A-I, A-II, and D than asymptomatic normal controls (1, 6, 15).

By investigating a larger population of A-I_M carriers we have clearly established that the previously recognized LP-A-I and LP-A-I:A-II particles (12) are significantly reduced in male and female carriers and that the percentage reduction of LP-A-I:A-II particles is greater than that of LP-A-I particles (60% vs. 40%). The LP-A-II particles have been identified for the first time as a minor apoA-containing lipoprotein family occurring at levels significantly lower than in normal controls (13, 32). The most characteristic compositional abnormalities detected in LP-A-I and LP-A-I:A-II particles of A-I_M carriers in comparison with those of normal controls included *1)* significantly higher percentages of triglycerides and phospholipids and lower percentages of free cholesterol and, especially, cholesteryl esters; *2)* a significantly lower percentage of apoD; and *3)* increased lipid/protein ratios observed primarily in lipoprotein families of male carriers. The composition of LP-A-II particles was similarly altered including a significantly reduced cholesteryl ester/triglyceride ratio, an increased lipid/protein ratio, and the presence of apoC-polypeptides and apoE in addition to apoA-II and apoD as apolipoprotein constituents of normal LP-A-II. Differences in the apolipoprotein composition of LP-A-I and LP-A-I:A-II particles between A-I_M carriers and normal controls were also reflected in the total concentrations of minor apolipoproteins associated with apoA-I-containing lipoproteins, among which the LP-A-I:A-II particles were affected to a greater and more significant extent that the LP-A-I particles.

This study has shown that the presence of apoA-I_M mutant also affects the apoB-containing lipoproteins. In contrast to decreased levels of apolipoproteins A-I and A-II and decreased levels of LP-A-I, LP-A-I:A-II, and LP-A-II particles, both male and female A-I_M carriers were shown to have significantly increased concentrations of apoB and the corresponding apoB-containing lipoproteins when compared to normal controls. Qualitatively, all major apoB-containing lipoproteins previously identified and characterized in normolipidemic and dyslipoproteinemic subjects (14, 22, 24, 25, 37) have also been detected in A-I_M carriers including cholesteryl ester-rich LP-B and triglyceride-rich LP-B:C, LP-B:C:E, and LP-A-II:B complex (LP-B_c) particles.

One of the main findings of this study was the disclosure of a characteristic sex-related difference in the concentration of apoA- and apoB-containing lipoproteins. Although there were no sex-related differences in the levels of total plasma cholesterol and triglycerides, female carriers had significantly higher concentrations of HDL-cholesterol, apolipoproteins A-I and A-II and, conse-

quently, higher concentrations of LP-A-I and LP-A-I:A-II particles than male carriers. Whereas the distribution of apoA-I between LP-A-I and LP-A-I:A-II particles was similar in female carriers (45% vs. 55%), male carriers had a significantly lower percentage of apoA-I in LP-A-I than in LP-A-I:A-II particles (29% vs. 71%). These sex-related differences seem to be consistent with those found in normal subjects showing that women have higher levels of apoA-I and higher levels and percentages of apoA-I in LP-A-I particles than men (16, 38). However, in A-I_M carriers, these differences are amplified. Judging from insignificant differences in the levels of VLDL-cholesterol, LDL-cholesterol, and minor apolipoproteins it would have been impossible to predict that the sex-related differences in the levels of apoA-containing lipoproteins also apply to major apoB-containing lipoproteins. Male carriers had higher concentrations of LP-B and LP-B:C:E particles and female carriers had higher concentrations of LP-B:C and LP-A-II:B complex particles. Due to relatively high levels of these two latter lipoprotein families, the combined concentrations of triglyceride-rich apoB-containing lipoproteins (LP-B_c) were higher in female than in male carriers (Table 5). An even greater difference in the levels of LP-B and LP-B_c particles was observed between two subpopulations of A-I_M carriers separated on the basis of their plasma levels of apoA-I (Table 6). The subpopulation with higher levels of apoA-I and, thus, higher levels of LP-A-I and LP-A-I:A-II particles, had lower concentrations of LP-B and higher concentrations of LP-B_c particles than the subpopulation with significantly reduced concentration of apoA-I. This inverse relationship between the levels of apoA- and apoB-containing lipoproteins has been shown to apply equally to LP-A-I and LP-A-I:A-II particles and, on the basis of present evidence, appears to be specific for A-I_M carriers. In comparison with other dyslipoproteinemias studied, A-I_M carriers had significantly reduced levels of LP-A-I:A-II, but similar levels of LP-A-I particles; whereas in other dyslipoproteinemic patients the correlation between LP-A-I and LP-B and LP-B_c was always opposite to that of LP-A-I:A-II, in A-I_M carriers the inverse correlation between LP-A-I and LP-B and LP-B_c paralled that of LP-A-I:A-II.

The available evidence suggests that gender plays a significant role in determining the proportions of normal and mutant monomeric and dimeric forms of apoA-I and, thus, the concentrations of apoA-containing lipoproteins in male and female A-I_M carriers. Previous analytical data (12) and recent results from one of our laboratories (G. Franceschini and C. R. Sirtori, unpublished results) suggest that female carriers have a higher normal/mutant apoA-I ratio than male carriers. On the other hand, based on the occurrence of characteristic size patterns of HDL_3 particles (7), male carriers have a higher dimer/monomer ratio of mutant apoA-I than female carriers. It has been shown previously that small HDL_{3b} particles (pattern I), thought to consist of apoA-I/apoA-II heterodimers, are more prevalent among male carriers, whereas large HDL_{3a} particles (pattern III), poorer in apoA-I_M heterodimers, occur more frequently among female carriers (7). Under such circumstances, due to a greater proportion of hypercatabolic monomeric mutant and higher content of apoA-I/apoA-II heterodimers, male carriers ought to have lower concentrations of both LP-A-I and LP-A-I:A-II particles and a lower LP-A-I/LP-A-I:A-II particle ratio than female carriers. In contrast, due to a greater percentage of normal than mutant apoA-I, female carriers ought to have moderately reduced levels of LP-A-I and LP-A-I:A-II particles and a similar distribution of apoA-I between these two HDL families. The occurrence of such lipoprotein particle patterns in male and female apoA-I_M carriers is corroborated by results shown in Table 3. Whether the coordinate relationship between the small HDL_{3b} particles and plasma apoB levels, detected in healthy, nonhyperlipidemic men and women (39), may also apply to A-I_M carriers as an explanation for their relatively high plasma apoB levels remains to be established.

It appears that in several hypoalphalipoproteinemic states moderately or markedly decreased concentrations of apoA-I are associated with either normal (40–42) or increased (22, 24, 36, 43) levels of apoB. Most frequently, a significant proportion of apoB-containing lipoproteins consists of intact or partially delipidized triglyceride-rich LP-B_c particles (22, 24, 36). A-I_M carriers seem to have a normally functioning lipolytic enzyme system with a normal or slightly reduced lipoprotein lipase (LPL) activity (1), normal levels of apoC-II, and a normal apoC-II/apoC-III weight ratio (Table 1). Recent studies have shown that LP-B:C and LP-B:C:E particles are effective substrates and LP-A-II:B complex particles a rather ineffective substrate for LPL (6). The increased concentration of LP-A-II:B complex particles in female carriers clearly represents one of the important factors contributing to their elevated vascular pool of triglyceride-rich lipoproteins; in contrast, the concentration of LP-A-II:B complex in male carriers is low. On the other hand, the levels of LP-B:C:E particles are low in female carriers and tend to be elevated in male carriers. It has been suggested that triglyceride-rich LP-B:C:E and phospholipid-rich LP-A-II:D particles might be the precursors of LP-A-II:B complex (22). If the sex-related difference in the levels of LP-A-I and LP-A-I:A-II particles (Table 3) also applies to the levels of LP-A-II particles, it is possible that female carriers may have higher concentrations of this minor lipoprotein family than male carriers. Consequently, female carriers should also have higher concentrations of LP-A-II:B complex and lower concentrations of LP-B:C:E particles than their male counterparts. An additional factor contributing to increased concentrations of

triglyceride-rich lipoproteins may be the structural alteration of LP-A-I and LP-A-I:A-II particles functioning as the acceptors of tissue lipids and surface components generated by lipolytic degradation of triglyceride-rich lipoproteins. There are significant differences between the normal apoA-I and monomeric and dimeric forms of apoA-I_M regarding their lipid-binding properties (8, 10) and fractional catabolic rates (9, 44); the apoA-I_M monomer has the highest lipid-binding affinity and the highest fractional catabolic rate followed by those of normal apoA-I and homo- and hetero-dimers of apoA-I_M. The occurrence of homo- and hetero-dimers of apoA-I_M may be responsible for a decreased lipoprotein surface of LP-A-I and, especially, LP-A-I:A-II particles and, thus, a reduced capacity to associate with minor apolipoproteins as evidenced by lower concentrations of apolipoproteins C-II, C-III, D, and E associated with LP-A-I:A-II particles (Table 4) and lower concentrations of apoC-III in heparin supernates (HS) of both male and female carriers (Table 2). On the other hand, one cannot exclude the possibility that increased levels of some apoB-containing lipoproteins, such as LP-B:C in female carriers and LP-B in male carriers, are caused by increased production rates of these lipoproteins as a general response to increased fractional catabolic rates of apoA-I and low HDL levels (45).

The concentration profiles of apoB-containing lipoprotein particles characterized in male carriers by high levels of cholesteryl ester-rich LP-B and in female carriers by high levels of intact or partially delipidized triglyceride-rich lipoproteins may be considered as equally atherogenic (17, 46, 47). However, despite increased levels of potentially atherogenic apoB-containing lipoproteins and reduced levels of nonatherogenic apoA-containing lipoproteins, no overt clinical manifestations of coronary artery disease have been detected among either the female or male A-I_M carriers (1, 6, 11, 15). Although the mechanism of antiatherogenic potential in A-I_M carriers has not been established, it appears to be closely associated with the presence of metabolically active monomeric apoA-I_M mutant (11). It is possible that LP-A-I particles and the changed lipid composition of both apoA-containing lipoproteins might play significant, if not pivotal, roles in these processes. Several recent studies have suggested that normal LP-A-I particles are metabolically more active than LP-A-I:A-II particles (48–50) including their capacity to acquire cholesterol from cultured peripheral cells (51, 52). Moreover, patients with angiographically documented coronary artery disease (53) or myocardial infarction (54) were found to have significantly lower levels of LP-A-I, but not LP-A-I:A-II particles, than corresponding controls. Despite the overall reduction in the levels of apoA-containing lipoproteins, the proportion of possibly anti-atherogenic LP-A-I particles remains unchanged in apoA-I_M male carriers and is significantly increased in female carriers when compared to normal controls. These findings also suggest that the putative antiatherogenic function of apoA-containing lipoprotein particles may depend to a greater extent on their qualitative rather than quantitative composition. In contrast to normal HDL particles, the lipid composition of LP-A-I and LP-A-I:A-II particles is characterized by higher percentages of triglycerides and phospholipids and lower percentages of free cholesterol and, especially, cholesteryl esters. The reduced cholesteryl ester/triglyceride ratio is most pronounced in HDL (1.8 in carriers vs. 9.5 in controls), but it is also characteristic of LDL (2.1 vs. 3.4) and VLDL (0.22 vs. 0.30). In HDL, the reduced cholesteryl ester/triglyceride ratio may be due to the low LCAT activity in apoA-I_M carriers (55) and/or the inability of apoA-I_M dimers to activate LCAT (56). Interestingly, genetic defects affecting LCAT, i.e., LCAT deficiency and fish-eye disease are both associated with a low incidence of coronary artery disease (57). Although the significantly reduced cholesteryl ester/triglyceride ratio in HDL particles may argue in favor of an increased exchange of cholesteryl esters for triglycerides between HDL and other major lipoprotein density classes (58), the actually decreased cholesteryl ester/triglyceride exchange detected in vitro during incubation of whole apoA-I_M plasma (8) and the lower percentage content of cholesteryl esters and a lower cholesteryl ester/triglyceride ratio in VLDL of apoA-I_M carriers do not seem to favor such mechanism of cholesteryl ester disposal. It appears, therefore, that the accelerated catabolism of particles containing monomeric apoA-I_M mutant may be considered as one of the potential mechanisms for an efficient disposal of free and esterified cholesterol in both the male and female apoA-I_M carriers.

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