Effects of gender and menopausal status on plasma lipoprotein subspecies and particle sizes

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Abstract The risk of coronary heart disease (CHD) is lower in women than in men, but increases in women after menopause. Some of the gender, age, and menopausal-related differences in CHD risk may relate to differences in lipoprotein subspecies. We therefore examined these subspecies in three groups of healthy subjects: premenopausal women (W, n = 72, mean age 41.2 ± 6.5), postmenopausal women (PMW, n = 74, 55.8 ± 7.4), and men (M, n = 139, 48.8 ± 10.7). We measured plasma levels of lipids, lipoprotein cholesterol, apolipoproteins A-I, A-IV, B, C-III, and E, and lipoprotein subspecies Lp A-I, Lp A-I:A-II, Lp B, Lp B:C-III, and Lp B:E, as well as LDL and HDL particle sizes. Our data indicate that women have significantly higher values of HDL-C, apoA-I, apoE, and Lp A-I; larger LDL and HDL particle sizes; and lower values of triglyceride, apoB, and Lp B:C-III particles than men, with no difference in Lp A-I:A-II. Postmenopausal status was associated with significantly higher values of total cholesterol, triglyceride, VLDL-C, and LDL-C; increased levels of apoB, C-III, and E; elevated values of Lp B, Lp B:C-III, and Lp B:E; and lower levels of HDL-C along with smaller HDL particle size. Moreover, we noted a strong correlation between LDL and HDL particle size. Our data are consistent with the concepts that male gender confers decreases in HDL subspecies due to lower Lp A-I levels; while postmenopausal status results in higher levels of all apoB-containing lipoproteins (Lp B, Lp B:C-III, and Lp B:E). The lipoprotein alterations associated with male gender and postmenopausal status would be expected to increase CHD risk.—**Li, Z., J. R. McNamara, J-C. Fruchart, G. Luc, J. M. Bard, J. M. Ordovas, P. W. F. Wilson, and E. J. Schaefer.** Effects of gender and menopausal status on plasma lipoprotein subspecies and particle sizes. *J. Lipid Res.* 1996. **37:** 1886–1896.

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Coronary heart disease (CHD) is a major cause of morbidity and mortality in the United States (1). Risk factors for CHD include: male gender, increased age, elevated plasma low density lipoprotein cholesterol (LDL-C), decreased high density lipoprotein cholesterol (HDL-C), high blood pressure, smoking, and diabetes mellitus (2–5). Gender differences in plasma levels of both LDL-C and HDL-C have been documented (6–9). Age and menopausal differences in these parameters have also been reported previously (10, 11). It has been suggested that these differences may, at least in part, explain the higher risk of CHD in men than in women, and in postmenopausal women than in premenopausal women.

It has been shown that both LDL and HDL are comprised of a number of different subspecies (12–19). These lipoprotein subspecies may be assessed by particle size and by apolipoprotein composition (13–22). We have used high resolution gradient gel electrophoresis methodology to assess both LDL and HDL particle size in whole plasma using Sudan black B staining and laser scanning densitometry (14–16). Different particle sizes have been documented within the LDL and HDL density regions, with up to 7 different particle sizes of LDL, and up to 14 different HDL particle sizes being observed (14–16). Most subjects have one to three major adjacent LDL bands, and four to five HDL bands. Decreased LDL and HDL particle sizes have been observed in CHD cases versus controls (21, 23–25). However, LDL size was found not to be an independent predictor of CHD in a case control study after controlling for other major CHD risk factors (smoking, hypertension, diabetes, high LDL-C, and low HDL-C) and the use of beta blockers (21).

Triglyceride-rich lipoproteins (TRL) of liver origin contain apolipoproteins (apo) B-100, C-I, C-II, C-III, and E, while other particles also of hepatic origin within TRL and intermediate density lipoproteins (IDL) contain

Abbreviations: apo, apolipoprotein; BMI, body mass index; CHD, coronary heart disease; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL-C, very low density lipoprotein cholesterol; Lp, lipoprotein; TRL, triglyceride-rich lipoproteins.

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TABLE 1. Gender differences in plasma lipids, apolipoproteins, and lipoprotein subspecies

	Females[a]	Males[a]	% Diff[b]
n	146	139	
Age (years)	48.7 ± 10.1	48.8 ± 10.7	
BMI (kg/m²)	25.6 ± 5.9	27.3 ± 4.0[c]	6.6
Glucose (mg/dl)	92.6 ± 25.8	98.9 ± 22.4[c]	6.8
TC (mg/dl)	209.4 ± 36.9	207.6 ± 35.4	
TG (mg/dl)	95.7 ± 59.3	119.2 ± 67.3[c]	24.6
VLDL-C (mg/dl)	38.9 ± 56.4	40.2 ± 49.6	
LDL-C (mg/dl)	131.2 ± 32.3	133.9 ± 31.5	
HDL-C (mg/dl)	58.7 ± 15.1	46.6 ± 11.9[c]	-20.6
TC/HDL-C ratio	3.8 ± 1.1	4.7 ± 1.3[c]	23.7
ApoA-I (mg/dl)	159.9 ± 35.1	141.0 ± 38.1[c]	-11.8
ApoA-IV (mg/dl)	17.0 ± 8.1	17.3 ± 5.0	
ApoB (mg/dl)	80.8 ± 24.8	91.2 ± 21.9[c]	12.9
ApoC-III (mg/dl)	3.4 ± 1.3	3.6 ± 2.0	
ApoE (mg/dl)	5.9 ± 2.6	5.2 ± 2.3[c]	-11.9
Lp A-I (mg/dl)	60.5 ± 19.8	44.2 ± 13.3[c]	-26.9
Lp A-I:A-II (mg/dl)	87.6 ± 22.4	87.5 ± 20.3	
Lp B (mg/dl)	109.1 ± 28.7	113.3 ± 29.3	
Lp B:C-III (mg/dl)	8.1 ± 5.8	9.9 ± 5.3[c]	22.2
Lp B:E (mg/dl)	19.6 ± 16.4	21.2 ± 16.3	
LDL size (nm)[d]	21.73 ± 0.2	21.51 ± 0.2[c]	-1.0
LDL score[e]	2.41 ± 0.9	3.30 ± 0.9[c]	36.9
Pattern A LDL (%)[f]	92	72[c]	-22.3
HDL size (nm)[d]	8.82 ± 0.3	8.53 ± 0.3[c]	-3.4
Large HDL (%)[f]	76	41[c]	-38.0
% $HDL_{2/3}$ ratio[g]	1.39 ± 1.5	0.60 ± 0.6[c]	57.6

[a]Mean ± SD.
[b]Percentage differences of means comparing males with females.
[c]Means are significantly different ($P < 0.05$).
[d]Estimated mean LDL and HDL particle sizes were calculated from results of 2–16% or 4–30% PAA gel, see Methods for details.
[e]A higher score represents a smaller particle size.
[f]Pattern A LDL data were estimated from our LDL scoring system, and large HDL was defined as % HDL_2 > 35% from the 4–30% PAA gel, see Methods section for details.
[g]Percentage of HDL_2 and HDL_3 was calculated from 4–30% PAA gel.

only apoB-100 and apoE. Within TRL of density <1.006 g/ml, two different lipoprotein subspecies can be measured by specialized ELISA methodology: lipoproteins containing apoB and apoC-III (Lp B:C-III), and those containing apoB and apoE (Lp B:E) (22). In our view, there are two types of particles within TRL: those containing apoB and apoE without apoC-III, and those containing apoB, apoE, and apoC-III. Therefore, apoB:C-III particles are a subset of Lp B:E particles, and in our view, they represent large, more triglyceride-rich TRL. Moreover, using apoE and apoA-I antisera for immunoprecipitation, one can remove all TRL and HDL from plasma, indicating that all TRL contain apoE (26). Within LDL, the major lipoprotein subspecies is a particle containing apoB-100 without other apolipoproteins (27). In contrast, within HDL, the two major subspecies are particles containing apoA-I and apoA-II (Lp A-I:A-II) as well as particles containing apoA-I without apoA-II (Lp A-I) (17, 18).

CHD patients have been reported to have lower levels of Lp A-I and Lp A-I:A-II, and higher levels of Lp B:E, in addition to decreased HDL-C and increased LDL-C

TABLE 2. Plasma lipids, apolipoproteins, and lipoprotein subspecies in pre- and postmenopausal women

	W[a]	PMW[a]	% Diff[b]
n	72	74	
Age (years)	41.2 ± 6.5	55.8 ± 7.4[c]	35.4
BMI (kg/m²)	24.5 ± 4.9	26.8 ± 6.6[c]	9.4
Glucose (mg/dl)	88.0 ± 14.4	97.1 ± 32.8[c]	10.3
TC (mg/dl)	193.4 ± 32.6	225.1 ± 34.3[c]	16.4
TG (mg/dl)	72.8 ± 35.0	117.9 ± 69.1[c]	62.0
VLDL-C (mg/dl)	29.3 ± 43.1	48.3 ± 65.8[c]	64.8
LDL-C (mg/dl)	117.6 ± 29.0	144.9 ± 29.7[c]	23.2
HDL-C (mg/dl)	61.0 ± 15.7	56.4 ± 14.1[c]	-7.5
TC/HDL-C ratio	3.3 ± 0.8	4.2 ± 1.2[c]	27.3
ApoA-I (mg/dl)	162.9 ± 32.9	157.1 ± 37.1	
ApoA-IV (mg/dl)	16.2 ± 4.4	17.8 ± 10.5	
ApoB (mg/dl)	71.7 ± 219.8	89.6 ± 26.2[c]	25.0
ApoC-III (mg/dl)	3.0 ± 1.2	3.6 ± 1.3[c]	20.0
ApoE (mg/dl)	5.3 ± 2.1	6.4 ± 2.9[c]	20.8
Lp A-I (mg/dl)	63.2 ± 19.4	58.0 ± 20.0	
Lp A-I:A-II (mg/dl)	86.7 ± 21.3	88.2 ± 23.4	
Lp B (mg/dl)	97.6 ± 23.7	118.9 ± 29.1[c]	21.8
Lp B:C-III (mg/dl)	5.7 ± 3.6	9.6 ± 6.4[c]	68.4
Lp B:E (mg/dl)	14.8 ± 11.4	23.6 ± 18.0[c]	59.5
LDL Size (nm)[d]	21.76 ± 0.2	21.70 ± 0.2	
LDL score[e]	2.28 ± 0.7	2.56 ± 1.0	
Pattern A LDL (%)[f]	97	88[a]	-8.4
HDL Size (nm)[d]	8.91 ± 0.3	8.73 ± 0.3[c]	-2.0
Large HDL (%)[f]	86	66[c]	-21.9
% $HDL_{2/3}$ ratio[g]	1.75 ± 1.9	1.03 ± 1.0[c]	-41.1

[a]W, premenopausal women; PMW, postmenopausal women.
[b]Percentage differences of means comparing pre- and postmenopausal women.
[c]Means are significantly different ($P < 0.05$).
[d]Estimated mean LDL and HDL particle sizes were calculated from results of 2–16% or 4–30% of PAA gel, see Methods for details.
[e]A higher score represents a smaller particle size.
[f]Pattern A LDL data were estimated from our LDL scoring system, and large HDL was defined as % HDL_2 >35% from the 4–30% PAA gel, see Methods section for details.
[g]Percentage of HDL_2 and HDL_3 was calculated from 4–30% PAA gel.

levels (28, 29). Our purpose was to investigate the effects of gender, age, and menopausal status on plasma lipids, apolipoproteins, lipoprotein subspecies as well as lipoprotein particle sizes, and to define the most important lipoprotein subspecies (Lp A-I, Lp A-I:A-II, Lp B, LpB:C-III and Lp B:E) associated with plasma concentrations of triglyceride, VLDL, LDL, and HDL cholesterol, as well as LDL and HDL particle size variability.

METHODS

Subjects

Subjects (n = 285) were randomly selected from examination cycle 3 of the Framingham Offspring Study (30). These subjects were divided into three groups according to their gender and menopausal status if female: 72 were premenopausal women, defined as having had menses in the past year (W, mean age: 41.2 ± 6.5 yr), 74 were postmenopausal women, defined as not having had menses in the past year (PMW, mean age: 55.8 ± 7.4 yr), and 139 were men (M, mean age: 48.8 ± 10.7 yr). No information about phase of menstrual cycle was obtained. Women who had undergone a hysterectomy with or without an oophorectomy were excluded from the analysis. All subjects were free living, and were not taking medications known to affect lipoprotein metabolism (such as lipid-lowering medications, estrogens, progestin, and thyroxine). None of the subjects had clinical or electrocardiographic manifestations of cardiovascular disease.

Lipid measurements

Blood was drawn in EDTA tubes to a final concentration of 0.15% after a 12–14 h fast. Plasma was isolated by centrifugation [2500 rpm (1000 *g*), at 4°C, 20 min], and aliquots were stored at -70°C for later determination of apolipoproteins and lipoprotein subspecies. The

VLDL fraction was separated from plasma by ultracentrifugation at a density of 1.006 g/ml. The HDL fraction was obtained by precipitation with dextran sulfate-Mg^{2+} (31). Total cholesterol (TC), triglyceride (TG), and HDL-C levels were measured enzymatically (Abbott Diagnostics, Dallas, TX) on an Abbott diagnostics ABA-200 bichromatic analyzer (32). VLDL-C and LDL-C levels were calculated by standard Lipid Research Clinics methodology as follows:

VLDL-C = TC -1.006 g/ml infranate cholesterol;
LDL-C = 1.006 g/ml infranate cholesterol -HDL-C.

Our laboratory is part of the Cholesterol Reference Method Laboratory Network and participates in the Centers for Disease Control and Prevention–National Heart, Lung, and Blood Institute Lipid Standardization Program. The within and between run coefficients of variation (CVs) for lipid measurements were all less than 5% (32).

Apolipoprotein assessments

Plasma levels of apoA-I and apoB were measured by non-competitive enzyme-linked immunosorbent assay (ELISA), using affinity-purified polyclonal antibodies. Between and within run CVs for those assays were ≤10%. The description of the anti-apoA-I and anti-apoB antibody preparation, the ELISA procedure, and calibration of the assays have been previously reported (33, 34). Plasma apoA-IV, apoC-III, and apoE levels were determined by ELISA as previously described, and between and within run CVs for these assays were ≤8% (35–38).

Lipoprotein subspecies determinations

Lipoprotein particles containing apoA-I without apoA-II (Lp A-I) and lipoprotein particles containing apoA-I and apoA-II (Lp A-I:A-II) were determined by differential electroimmunoassay. This method allows for the direct measurement of Lp A-I by using a large excess of anti-apoA-II antibodies; Lp A-I:A-II particles are retained in one peak and Lp A-I migrates as a second peak (18). Lipoprotein particles containing apoB only (Lp B) were separated by affinity chromatography on an anti-apoB-100-Sepharose column using monoclonal antibodies that recognize Lp B, but not Lp B:C-III or Lp B:E. After this fraction was eluted from the column the concentration of apoB was measured by ELISA as previously reported (39). Lipoprotein particles containing apoB and apoC-III (Lp B:C-III), and lipoproteins containing apoB and apoE (Lp B:E) were quantified by a two-site ELISA method, as previously reported (22). The ELISA for Lp B:C-III and Lp B:E measurements were carried out as follows: plasma samples were incubated in plates precoated with purified antibodies against apoC-III, or apoE; and after washing, the plates were incubated again with peroxidase-labeled anti-apoB, after which the levels of Lp B:C-III or Lp B:E were determined respectively (22). CVs for all of these assays both within and between run were ≤10%.

LDL and HDL particle size assessment

LDL and HDL particle sizes were examined by using 2–16% or 4–30% polyacrylamide (PAA) gradient gel electrophoresis as previously described (14–16). Nondenaturing 2–16% and 4–30% PAA gradient gels were obtained from Pharmacia (Piscataway, NJ). Briefly, plasma was used to assess LDL and HDL particle size, Sudan Black B solution was used to stain the particles in the PAA gels separated by electrophoresis, and a 50% solution of ethylene glycol monoethyl ether was used to destain the gels. Gels were scanned at 633 nm with an LKB laser densitometer, and data were integrated by GSXL software. We have previously documented that freezing plasma once at -80°C does not affect the particle size analysis. LDL and HDL score were obtained as previously described; CVs for examining LDL score, HDL score, and percentage of HDL_2 were 4.1%, 3.9%, and 12.1%, respectively (14, 15).

In our current study, we calculated the mean LDL and HDL particle sizes by the following equation:

$$\text{Mean particle size (nm)} = \sum_{i=1}^{n=8 \text{ or } 14} (X_i * Y_i)$$

where i is the designation of an LDL or HDL band size obtained from the PAA gel, n = 8 for LDL and n = 14 for HDL, X is the estimated diameter (nm) for an LDL or HDL band, and Y is the fraction of total area for that band. The mean particle size is the estimated mean diameter for the whole spectrum of LDL or HDL particles.

The estimated diameters for the LDL bands obtained from 2–16% PAA gels were based on the results of our collaborative work using electron microscopy (personal communication with Dr. James Otvos, North Carolina State University, and Dr. Lawrence Rudel, Bowman Gray School of Medicine) and GGE measurement from this laboratory (40) and are defined as LDL_1 = 22.1 nm, LDL_2 = 21.7, LDL_3 = 21.7, LDL_4 = 21.3, LDL_5 = 21.0, LDL_6 = 20.8, LDL_7 = 20.7, LDL_8 = 19.4, respectively.

The estimated diameters for the HDL bands obtained from 4–30% PAA gels were previously reported from this laboratory, and are shown here for completeness, as HDL-1 = 12.46 nm, HDL-2 = 11.74, HDL-3 = 11.25, HDL-4 = 10.96, HDL-5 = 10.55, HDL-6 = 10.00, HDL-7 = 9.57, HDL-8 = 9.24, HDL-9 = 8.90, HDL-10 = 8.73, HDL-11 = 8.53, HDL-12 = 8.30, HDL-13 = 8.14, and HDL-14 < 7.86, respectively (14).

For comparison of our LDL and HDL sizes and scores with other commonly used nomenclature, LDL scores were combined into LDL patterns A and B: an LDL

score <3.5 was considered equivalent to LDL pattern A, and a score ≥3.5 was equated to LDL pattern B (41); HDL-1 to HDL-10 have previously been defined as HDL_2 and HDL-11 to HDL-14 as HDL_3 (14), an HDL_2 percentage >35% was designated as large HDL, and a percentage ≤35% was designated as small HDL.

Statistics analysis

Data were entered and stored in a VAX 11/785 (Digital Equipment Corporation, Maynard, MA), using the RS/1 software package (BBN Software, Cambridge, MA). Statistical Analysis System (SAS) software (SAS Institute, Inc., Cary, NC) was used to analyze the data. A logarithmic transformation was applied to variables that were not normally distributed to approximate normal distribution before testing for significant difference. These variables were: plasma glucose, triglycerides, VLDL-C, HDL-C, apoA-I, apoB, apoC-III, apoE, and all lipoprotein subspecies values. ANOVA was used to test for significant difference of mean values among the test groups. Pearson correlation coefficient analysis was performed to assess the correlations between all parameters among test groups. A stepwise regression analysis was used to assess the contribution of lipoprotein subspecies (Lp A-I, LpA-I:A-II, Lp B, Lp B:C-III, and LpB:E) to variability in VLDL-C, LDL-C, HDL-C, TG, and mean LDL and HDL particle sizes.

RESULTS

Table 1 shows the gender difference for age, body mass index (BMI), plasma values of glucose, lipids, apolipoproteins and lipoprotein subspecies, as well as data of LDL and HDL particle sizes. Compared with women, men in this population had significantly higher BMI, plasma levels of glucose, TC/HDL ratio, apoB, and Lp B:C-III ($P < 0.05$), and significantly lower levels of HDL-C, apoA-I, apoE, and Lp A-I ($P < 0.05$); Moreover, men had smaller LDL and HDL particle size and a lower % HDL_2/HDL_3 ratio, less pattern A LDL and less large HDL than women ($P < 0.05$). There were no significant gender differences in age, plasma levels of TC, VLDL, LDL-C, apoA-IV, apoC-III, Lp A-I:A-II, Lp B, and Lp B:E between the male and female groups studied (see Table 1).

Data on plasma lipids, apolipoproteins and lipoprotein subspecies in pre- and postmenopausal women are provided in **Table 2.** Postmenopausal women were significantly older, and had higher BMI, plasma levels of glucose, TC, TG, VLDL-C, LDL-C, TC/HDL-C ratio, apoB, C-III, and E, as well as Lp B, Lp B:C-III, and Lp B:E than premenopausal women ($P < 0.05$). Postmenopausal women had modestly, but significantly, lower HDL-C than premenopausal women ($P < 0.05$). These changes were accompanied by smaller HDL particles, a lower % HDL_2/HDL_3 ratio, less pattern A LDL, and less large HDL. Plasma levels of apoA-I, A-IV, Lp

TABLE 3. HDL particle distribution (%) in all subjects

	W	PMW	M	Other Designations
n	72	74	139	
HDL-1	0.1 ± 0.6	0.1 ± 0.3	0.1 ± 0.4	HDL_{2b}
HDL-2	0.3 ± 1.1	0.1 ± 0.5	0.1 ± 0.3	HDL_{2b}
HDL-3	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.2	HDL_{2b}
HDL-4	0.3 ± 1.2	0.4 ± 1.3	0.3 ± 1.4	HDL_{2b}
HDL-5	3.9 ± 7.6	2.8 ± 4.8	1.7 ± 3.1	HDL_{2b}
HDL-6	16.3 ± 15.2	10.3 ± 13.1	5.9 ± 7.9	HDL_{2b}
HDL-7	8.3 ± 12.8	5.8 ± 9.2	2.4 ± 5.3	HDL_{2b}
HDL-8	6.8 ± 9.9	5.3 ± 7.1	4.1 ± 6.9	HDL_{2a}
HDL-9	12.3 ± 11.4	14.6 ± 13.8	13.1 ± 11.1	HDL_{2a}
HDL-10	5.8 ± 11.8	3.1 ± 8.2	3.7 ± 9.3	HDL_{2a}
HDL-11	14.5 ± 12.2	18.0 ± 12.9	14.5 ± 13.9	HDL_{3a}
HDL-12	17.0 ± 14.7	21.0 ± 17.2	29.3 ± 20.3	HDL_{3b}
HDL-13	13.5 ± 13.9	16.8 ± 14.7	20.7 ± 19.2	HDL_{3b}
HDL-14	0.9 ± 1.9	1.6 ± 2.9	4.1 ± 7.4	HDL_{3c}

Values given as mean percentage ± SD of each HDL band in three examined groups; W, premenopausal women; PMW, postmenopausal women; M, males.

TABLE 4. Distribution of plasma lipids and lipoprotein subspecies in pre- and post-menopausal women and men dichotomized according to predominantly large or small HDL

	W[a] n = 72	PMW[a] n = 74	M[a] n = 139	P W:PMW	P W:M	P PMW:M
Large HDL[b]						
%	86	66	41			
BMI (kg/m²)	23.8 ± 4.2	25.5 ± 6.3	26.3 ± 4.1	0.05	0.001	-
TC (mg/dl)	193.2 ± 28.8	225.0 ± 32.3	209.6 ± 34.9	0.001	0.01	0.02
TG (mg/dl)	68.4 ± 29.0	106.8 ± 72.0	95.3 ± 55.4	0.001	0.001	-
LDL-C (mg/dl)	116.6 ± 24.8	140.3 ± 27.5	134.8 ± 31.4	0.001	0.002	-
HDL-C (mg/dl)	63.2 ± 15.3	61.7 ± 13.7	53.7 ± 12.2	-	0.001	0.001
Lp A-I (mg/dl)	64.9 ± 19.5	60.7 ± 22.0	48.5 ± 14.7	-	0.001	0.002
Lp A-I:A-II (mg/dl)	86.9 ± 22.2	88.1 ± 24.3	91.5 ± 17.8	-	-	-
Lp B (mg/dl)	97.8 ± 22.8	117.7 ± 27.0	107.2 ± 22.5	0.001	-	-
Lp B:C-III (mg/dl)	5.6 ± 3.6	9.2 ± 7.3	8.4 ± 4.4	0.002	0.001	-
Lp B:E (mg/dl)	14.6 ± 11.8	19.9 ± 19.5	18.3 ± 10.9	-	0.02	-
Mean LDL size (nm)	21.78 ± 0.2	21.75 ± 0.2	21.63 ± 0.2	-	0.001	0.001
Mean HDL size (nm)	8.99 ± 0.3	8.88 ± 0.2	8.78 ± 0.2	0.01	0.001	0.01
Small HDL[b]						
%	14	34	59			
BMI (kg/m²)	28.5 ± 6.6[c]	29.5 ± 6.7[c]	28.0 ± 3.8[c]	-	-	-
TC (mg/dl)	194.7 ± 52.2	225.3 ± 38.5	206.2 ± 35.8	0.02	-	0.02
TG (mg/dl)	99.7 ± 54.6	139.5 ± 59.4[c]	135.8 ± 70.2[c]	0.03	0.04	-
LDL-C (mg/dl)	123.9 ± 49.8	154.0 ± 32.1	133.3 ± 31.8	0.01	-	0.005
HDL-C (mg/dl)	47.7 ± 11.5[c]	45.8 ± 7.5[c]	41.6 ± 8.9[c]	-	-	0.04
Lp A-I (mg/dl)	51.7 ± 14.7	53.2 ± 15.3	40.2 ± 10.4[c]	-	0.04	0.001
Lp A-I:A-II (mg/dl)	85.7 ± 13.4	89.2 ± 22.0	84.5 ± 21.6	-	-	-
Lp B (mg/dl)	96.5 ± 30.1	121.2 ± 33.2	118.3 ± 33.2	0.04	0.03	-
Lp B:C-III (mg/dl)	6.3 ± 3.7	10.1 ± 4.7	10.8 ± 5.7	0.04	0.03	-
Lp B:E (mg/dl)	16.5 ± 9.9	30.3 ± 15.7[c]	23.3 ± 19.0	0.02	-	0.02
Mean LDL size (nm)	21.64 ± 0.2[c]	21.56 ± 0.2[c]	21.43 ± 0.3[c]	-	0.005	0.02
Mean HDL size (nm)	8.42 ± 0.1[c]	8.44 ± 0.1[c]	8.35 ± 0.1[c]	-	-	-

[a]W, premenopausal women; PMW, postmenopausal women; M, males.
[b]Large HDL was defined as % HDL_2 >35, and small HDL was defined as % HDL_2 ≤35 by GGE measurement, see Methods section for details.
[c]Significant differences between large HDL and small HDL group ($P < 0.05$).

A-I and Lp A-I:A-II were not significantly different in these two groups of women (Table 2).

We did a comparison of age differences in men (≤50 versus >50 age group) and found that older men had higher plasma levels of glucose (14% higher) and Lp B (14% higher) than younger men ($P < 0.05$); no other significant differences were observed (data not shown). Therefore, all males have been placed in a single group to study the effects of gender. **Table 3** provides data on the distribution of HDL particles for the entire spectrum of HDL bands observed in 4–30% PAA gels. It can be readily seen that only a very low percentage of HDL in all study groups was found in HDL-1 to HDL-5. HDL-6 represented a major band, especially in women, and HDL-9 represented a major band in both men and women, as did HDL-11, HDL-12, and HDL-13. HDL-12 and HDL-13 were especially prominent bands in men accounting for over 50% of HDL.

Table 4 provides data for plasma lipids, lipoprotein subspecies, and mean LDL and HDL sizes in these three study groups after dichotomization based on predominance of large or small HDL. In the large HDL groups, premenopausal women had the largest HDL size and lowest levels of TC, followed by postmenopausal women and then men ($P < 0.05$). Within this group, premenopausal women had lower values of TG, LDL-C, Lp B:C-III, than did postmenopausal women and men ($P < 0.05$). Women had higher values of HDL-C, Lp A-I, LDL size than men ($P < 0.05$). Moreover, postmenopausal women had the highest levels of apoB-containing lipoproteins. In the small HDL groups, postmenopausal women had the highest levels of TC, LDL-C, Lp B:E among these three study groups ($P < 0.05$). Men and postmenopausal women had higher levels of TG, Lp B, and Lp B:C-III than premenopausal women ($P < 0.05$). Women had higher HDL-C and men had smaller LDL size ($P < 0.05$). When comparing subjects with large versus small HDL particle size in the same gender and menopause group, we observed that in all groups, subjects with large HDL had significantly lower values of BMI and higher levels of HDL-C, and larger LDL size ($P < 0.05$). In addition, in the postmenopausal women group, subjects with large HDL also had lower levels of TG and Lp B:E than those with small HDL ($P < 0.05$);

TABLE 5. Correlation matrix of mean HDL particle size with plasma lipids, lipoprotein subspecies, and mean LDL particle size in three examined groups

	Premenopausal Women		Postmenopausal Women		Men	
	Corr	*P*	Corr	*P*	Corr	*P*
BMI	-0.40	0.001	-0.27	0.02	-0.34	0.001
LDL	-0.23	-	-0.23	-	-0.06	-
TG	-0.41	0.001	-0.45	0.001	-0.47	0.001
HDL	0.66	0.001	0.71	0.001	0.60	0.001
Lp A-I	0.40	0.003	0.27	0.04	0.29	0.004
Lp A-I:A-II	-0.04	-	-0.10	-	0.10	-
Lp B	-0.35	0.01	0.04	-	-0.25	0.009
Lp B:C-III	-0.36	0.03	-0.28	0.04	-0.23	0.03
Lp B:E	-0.03	-	-0.43	0.001	-0.09	-
LDL size	0.36	0.002	0.47	0.001	0.48	0.001

and men in the large HDL group had lower levels of TG and Lp A-I than those in the small HDL group ($P < 0.05$).

Data providing correlations for biochemical parameters are given in **Table 5.** In all groups the mean HDL size was positively correlated with HDL-C, Lp A-I and mean LDL size, and also negatively correlated with BMI, TG and Lp B:C-III ($P < 0.05$). Moreover, mean HDL size was negatively correlated with Lp B in premenopausal women and men, and this parameter was negatively correlated with Lp B:E in the postmenopausal women group only ($P < 0.05$). LDL size and HDL size were significantly correlated with each other in all study groups (Table 5).

To further analyze the interrelationship between BMI, lipoprotein subspecies of Lp A-I, LpA-I:A-II, Lp B, LpB:C-III, and Lp B:E with triglycride, VLDL, LDL, and HDL cholesterol, and particle sizes, we did stepwise regression analyses among these parameters. **Table 6** shows the results of these associations in these gender and menopausal groups. In both men and women, not surprisingly, Lp B was the most important lipoprotein subspecies associated with LDL-C variability. In addition, Lp B:C-III was positively associated with LDL-C variability in premenopausal women, while Lp A-I was negatively associated with LDL-C in postmenopausal women. Lp A-I:A-II was positively associated with LDL-C in men ($P < 0.05$). Lp B:C-III was the only or major lipoprotein subspecies to be associated with mean LDL size in all groups, with higher values being associated with smaller LDL particle size ($P < 0.05$). Lp B was also negatively associated with LDL size in postmenopausal women ($P < 0.05$), and Lp A-I was positively associated with LDL size in men ($P < 0.05$). Lp A-I explained most of the variability in HDL-C levels in all three groups of men and women. BMI was also negatively correlated with HDL-C in premenopausal women and men. However, in postmenopausal women, TG-rich lipoproteins (Lp B:C-III, Lp B:E) were negatively and significantly associated with HDL-C variability ($P < 0.05$). Lp A-I was the only lipoprotein subspecies contributing to mean HDL size in premenopausal women ($P < 0.01$). In contrast, Lp B:E explained the only variability in HDL particle size in postmenopausal women with a negative association ($P < 0.01$). In men, BMI and Lp B:C-III were negatively associated with HDL size, while Lp A-I was positively associated with HDL size. TRL subspecies Lp B:C-III and Lp B:E were associated with plasma levels of VLDL-C and TG in all three groups, with the strongest association being observed with Lp B:C-III ($P < 0.05$, Table 6).

DISCUSSION

It has been documented that LDL cholesterol, apoB, and triglyceride levels are higher in males than in premenopausal females. These parameters increase after menopause in females; women at all ages have higher HDL cholesterol levels than men (7, 10, 11, 42–45). Elevated LDL cholesterol and decreased HDL cholesterol levels are clearly coronary heart disease (CHD) risk factors (2–4, 46). Therefore, the risk of CHD is higher in men than in women (6–8), and the CHD risk increases in women after menopause (7, 10, 11, 42–45). Moreover, it has been reported that CHD patients have significantly higher values of apoB, and Lp B:E, and lower values of Lp A-I and Lp A-I:A-II lipoprotein subspecies than control subjects (29, 47, 48). Part of the gender- and menopausal status-related differences in CHD risk may relate to alterations in lipoprotein subspecies, as well as to the changes in plasma lipids (17, 19, 23, 28, 29).

The data from this study show that women, regardless of menopausal status, have significantly different lipid, apoprotein, and lipoprotein profiles than men. Women in this sample had lower values of BMI, TG, TC/HDL ratio, apoB, E, Lp B:C-III and higher levels of HDL-C, apoA-I, Lp A-I, as well as larger mean LDL and HDL sizes and a higher % HDL_2/HDL_3 ratio than men. These

TABLE 6. Stepwise regression analysis of lipoprotein subspecies association with triglyceride, VLDL, LDL, and HDL cholesterol concentration and LDL and HDL particle size

	Premenopausal Women		Postmenopausal Women		Men	
1. LDL cholesterol model						
	Lp B	0.32[b]	Lp B	0.34c	Lp B	0.49[c]
	Lp B:C-III	0.14[a]	-Lp A-I	0.12b	Lp A-I:A-II	0.04[a]
2. LDL size model						
	-Lp B:C-III	0.17[a]	-Lp B:C-III	0.21[b]	-Lp B:C-III	0.18c
			-Lp B	0.09[a]	Lp A-I	0.14[b]
3. HDL cholesterol model						
	Lp A-I	0.65[c]	Lp A-I	0.20[c]	Lp A-I	0.62c
	-BMI	0.07[a]	-Lp B:C-III	0.16[b]	-BMI	0.03[a]
			Lp A-I:A-II	0.08[a]		
			-Lp B:E	0.08[a]		
4. HDL size model						
	Lp A-I	0.37[c]	-Lp B:E	0.14[a]	-BMI	0.17[c]
					Lp A-I	0.09[b]
					-Lp B:C-III	0.05[a]
5. VLDL cholesterol model						
	Lp B:C-III	0.20[b]	Lp B:C-III	0.17c	Lp B:C-III	0.30[c]
					Lp B:E	0.06[a]
6. Triglyceride model						
	Lp B:C-III	0.46[c]	Lp B:C-III	0.31[c]	Lp B:C-III	0.40[c]
	Lp B:E	0.16[b]	Lp B:E	0.08b	Lp B:E	0.04[a]

Lipoprotein subspecies (Lp A-I, Lp A-I:A-II, Lp B, Lp B:C-III, Lp B:E) and BMI variables were put into the test models to examine the interrelationship of these variables with variabilities of plasma concentrations of VLDL, LDL, and HDL cholesterol, and triglyceride, as well as LDL and HDL particle size in these three groups. Minus sign in front of parameter indicates negative association with the model parameter.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

results indicate that gender affects these parameters, and this effect is independent of age and menopausal effects (15, 49, 50). Presumably these differences are due to the different levels of circulating sex hormones, specifically estrogens and androgens in women versus men. It has been reported that women have higher production rates of apoA-I, the major HDL apoprotein, than do men, and that levels of apoA-I and production rates of apoA-I and Lp A-I can be increased with estrogen administration (51–53).

Lipoproteins containing apoB only (Lp B) were significantly lower in younger men than in older men in our study (data not shown), indicating an age effect on this lipoprotein subspecies in males. It has been reported that plasma levels of apoB, the only apolipoprotein present in Lp B particles, are associated with age in the Framingham Offspring population study and other studies (41–45, 54, 55). ApoB-100 kinetic studies have shown that the catabolic rate of LDL apoB is impaired with aging and that VLDL apoB secretion is increased and VLDL apoB catabolism is impaired with aging (56, 57). Decreased activity of the LDL receptor with age may well explain this effect.

Elevated levels of HDL cholesterol, apoA-I, and Lp A-I lipoprotein subspecies have been reported to be negatively associated with risk of CHD (29, 47, 58). Our data indicate that premenopausal women not only have significantly lower values of BMI, triglyceride, LDL-C, TC/HDL ratio, apoB, and all apoB-containing lipoprotein particles, but also have higher levels of HDL-C and Lp A-I, larger mean HDL size, and a higher % HDL_2/HDL_3 ratio than postmenopausal women and men (Tables 1, 2). These data suggest that sex hormones affect lipoprotein subspecies. The higher estrogen levels in premenopausal women may play a significant role in these differences. Moreover, our data clearly indicate that after menopause, women have significantly higher levels of all apoB-containing lipoproteins (Lp B, Lp B:C-III and Lp B:E). These alterations are not only associated with increased levels of LDL cholesterol and triglyceride, but also with decreased values of HDL cholesterol, mean HDL size, and % HDL_2/HDL_3 ratio (Tables 2, 5), although the levels of apoA-I and Lp A-I are similar in both female groups. These overall changes after menopause will increase the risk for CHD, and these changes can be corrected, at least in part, by estrogen replacement. LDL cholesterol and apoB levels have been shown to decrease significantly after estrogen replacement in dyslipidemic postmenopausal women, while HDL cholesterol and Lp A-I levels increased (52, 53, 59, 60).

HDL cholesterol concentration and mean HDL size are significantly correlated with each other, and each of

these parameters is inversely correlated with triglyceride levels in humans (14, 61, 62). These associations were also observed in the current study, with the highest levels of HDL cholesterol and the largest HDL particles being observed in premenopausal women with the lowest levels of triglycerides. It should be noted that HDL free cholesterol content and concentration was the most important HDL constituent affecting HDL particle size in our previous studies (14). We have observed the same phenomenon for LDL particle size (40). It has been reported that the rate of apoA-I catabolism is the most important factor regulating apoA-I and HDL cholesterol levels (51, 63–65), and the rate of apoA-I catabolism may also regulate HDL particle size and Lp A-I levels, because apoA-I is the major apolipoprotein constituent of Lp A-I, and Lp A-I is the major lipoprotein species within the large HDL density region.

Women have lower hepatic lipase activity than men, and as estrogen administration lowers hepatic lipase activity in women (59, 60), one might hypothesize that the gender difference in HDL levels relates to alterations in catabolism of HDL. However, the data indicate that the gender difference in apoA-I and Lp A-I is due to increased production rate in women, and that estrogen administration increases apoA-I and Lp A-I production, but not apoA-II and Lp A-I:A-II production (51–53). It should be noted that postmenopausal women had significantly higher levels of Lp B:C-III and Lp B:E, and these elevations were negatively associated with HDL cholesterol levels and mean LDL and HDL particle sizes ($P < 0.05$, Tables 2, 5, and 6), indicating that these triglyceride-rich lipoprotein subspecies, especially Lp B:E, are involved in HDL metabolism after menopause.

The conclusions of our studies are that: *1)* HDL particle size and Lp A-I levels are significantly greater in women than in men; *2)* Lp A-I levels, but not Lp A-I/A-II levels, are a significant determinant of apoA-I and HDL cholesterol concentrations, and HDL particle size; *3)* BMI are negatively correlated with HDL cholesterol levels in premenopausal women and men, and BMI are also negatively correlated with HDL size in men; *4)* postmenopausal women have significantly higher levels of triglyceride, LDL cholesterol, and all apoB-containing lipoproteins (Lp B, Lp B:C-III, and Lp B:E) than premenopausal women; *5)* levels of Lp B:C-III are inversely correlated with mean LDL size in all groups; and *6)* levels of Lp B:E are negatively correlated with HDL particle size in postmenopausal women.

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