

Factors associated with low and elevated plasma high density lipoprotein cholesterol and apolipoprotein A-I levels in the Framingham Offspring Study

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Abstract A decreased high density lipoprotein (HDL) cholesterol level (<35 mg/dl) has been shown to be a significant independent risk factor for coronary heart disease (CHD). Moreover, increased HDL cholesterol levels (≥ 60 mg/dl) are associated with a decreased CHD risk. Levels of HDL cholesterol and apoA-I, the major protein constituent of HDL, were measured in plasma from fasting participants in the Framingham Offspring Study (1,584 men and 1,639 women, mean age 49 ± 10 years). In this population, an HDL cholesterol value <35 mg/dl was observed in 18.2% of men and 3.8% of women, and these subjects had mean apoA-I levels of 104 and 106 mg/dl, respectively, and triglyceride levels of 234 and 261 mg/dl, respectively. CHD was observed in 14.2% of men and 14.5% of women in this category. An HDL cholesterol level ≥ 60 mg/dl was observed in 11.7% of men and 39.3% of women, and these subjects had mean apoA-I levels of 182 and 185 mg/dl, respectively, and mean triglyceride levels of 81 and 75 mg/dl, respectively. CHD was noted in 2.7% of men and 1.9% of women in this category.

HDL cholesterol levels were much more strongly related to triglycerides ($r = -0.54$ in men and -0.47 in women) than was apoA-I ($r = -0.26$ in men and -0.13 in women). The relationship between plasma HDL cholesterol and triglyceride levels was not linear. In both men and women, triglycerides, body mass index (BMI), and alcohol intake contributed significantly to HDL cholesterol and apoA-I variability.—Schaefer, E. J., S. Lamon-Fava, J. M. Ordovas, S. D. Cohn, M. M. Schaefer, W. P. Castelli, and P. W. F. Wilson. Factors associated with low and elevated plasma high density lipoprotein cholesterol and apolipoprotein A-I levels in the Framingham Offspring Study. *J. Lipid Res.* 1994. 35: 871-882.

Supplementary key words cholesterol • triglycerides • coronary heart disease • body mass index • diabetes mellitus • hypertension

A great deal of research interest over the past two decades has focused on high density lipoprotein (HDL) particles because of the association between reduced levels of HDL cholesterol and an increased risk of coronary heart disease (CHD) (1-6). Data from several epidemio-

logical studies have indicated that for each 1 mg/dl increase in HDL cholesterol levels the risk of CHD decreases by approximately 2-3% (1, 4, 5). We have previously shown that decreased plasma HDL cholesterol levels (<35 mg/dl) are the most common lipid abnormality found in CHD patients (7). Recently, the National Cholesterol Education Program (NCEP) Adult Treatment Panel II has defined a plasma HDL cholesterol value <35 mg/dl as a major and independent risk factor for CHD and a value ≥ 60 mg/dl as being protective against CHD (8). According to these NCEP guidelines, both decreased and elevated plasma HDL cholesterol levels should be taken into account when implementing therapy for increased low density lipoprotein (LDL) cholesterol levels.

HDL particles are involved in the reverse cholesterol transport pathway, and in vitro experiments have shown that HDL particles may promote the efflux of cholesterol from peripheral cells (9, 10). Apolipoprotein A-I (apoA-I) is the most important structural component of HDL, and elevated levels of expression of human apoA-I have been associated with resistance to diet-induced atherosclerosis in transgenic mice (11). Subjects who lack the expression of the apoA-I gene, as a result of deletions, rearrangements, or point mutations in the DNA region coding for apoA-I, have virtually no HDL in their plasma, and develop premature coronary heart disease (CHD) (12-14). Common genetic disorders associated with reduced levels

Abbreviations: CHD, coronary heart disease; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins.

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of apoA-I and HDL cholesterol include familial hypertriglyceridemia with hypoalphalipoproteinemia (or familial dyslipidemia), familial combined hyperlipoproteinemia with hypoalphalipoproteinemia, and familial hypoalphalipoproteinemia (3, 7, 15, 16). These disorders have been observed in 14.7%, 11.7%, and 4.0%, respectively, of families in which the proband had premature CHD (16).

Some studies have indicated that plasma apoA-I levels may be better indicators of CHD risk than HDL cholesterol levels (17–19). However, these findings have not been confirmed by prospective studies indicating that HDL cholesterol levels, and the total cholesterol to HDL cholesterol ratio, are the best predictors of CHD (20).

No information is presently available on apoA-I levels in large populations. In the present study we report normal ranges for HDL cholesterol and apoA-I plasma levels in the Framingham Offspring Study. We also report plasma lipoprotein profiles and characteristics of male and female subjects with low and high plasma levels of HDL cholesterol and apoA-I. In addition, we have evaluated the effect of age, gender, and menopausal status on HDL cholesterol and apoA-I levels, and determined the relationship between these plasma parameters and CHD in a case-control fashion.

MATERIALS AND METHODS

Preparation and isolation of anti-apoA-I antibody and enzyme-linked immunosorbent assay (ELISA)

Human HDL in the density range of 1.080–1.100 g/ml were obtained by preparative ultracentrifugation from pooled plasma of fasting individuals. Apolipoprotein A-I was separated from the HDL fraction by column chromatography. Injections of 5–10 mg of apoA-I protein with complete Freund's adjuvant were administered to goats four times over a 4-month period. To determine the specificity of the anti-apoA-I antibody, goat serum was tested by double radioimmunoassay and two-dimensional electrophoresis using total plasma, human albumin, isolated apolipoproteins A-I, A-II, C-II, C-III, and isolated HDL and LDL (which contained mostly apoB-100). Immunoreactivity was detected in whole plasma and isolated apoA-I and HDL only. Western blotting analysis demonstrated lack of cross-reactivity of the anti-apoA-I antibody with other apolipoproteins. The anti-apoA-I specific antibody was isolated from the total goat IgG fraction by affinity chromatography using a Sepharose 4B gel coupled with a purified apoA-I column (21).

The primary standard for our assay was purified apoA-I whose protein content was determined by the method of Lowry et al. (22) as well as by amino acid analysis. Secondary plasma standard samples were prepared in our laboratory by pooling plasma from different individuals and storing multiple aliquots at -80°C . The IUIS-WHO

apoA-I serum reference material (CDC #1883) was measured with our assay and an apoA-I concentration of 112 mg/dl, corresponding to the 1985 consensus value, was obtained. (Since then, the consensus value for that serum reference material has been changed to 124 mg/dl. Therefore, should the 124 mg/dl value be confirmed, a correlation factor of 1.1071 (multiplication) needs to be applied to our apoA-I data, as well as a correction factor for plasma versus serum).

Our ELISA procedure for apoA-I is essentially identical to that previously described by our laboratory for the measurement of apoB (23), with the exception that plasma samples were diluted 1:60,000 in a phosphate-buffered saline (PBS) solution containing 0.125% Tween. Briefly, microtiter plates (Nunc Immunoplate I, Nunc, Denmark) were coated with immunopurified anti-apoA-I antibody (concentration of 2.6 $\mu\text{g}/\text{ml}$). Plasma samples, diluted 1:60,000 in 1 \times PBS containing 0.125% Tween and 0.5% bovine serum albumin, were incubated overnight at room temperature in coated wells. Unbound material was then removed by three washes with the washing solution (PBS containing 0.05% Tween). After incubation at room temperature for 5 h with the alkaline phosphatase-conjugated anti-apoA-I antibody, plates were washed three times with the washing solution. The substrate (*p*-nitrophenyl phosphate, 1 mg/ml in 0.1 M glycine buffer) was then added. The color reaction was stopped after 20 min incubation by the addition of NaOH to a final concentration of 0.2 M. The absorbance was read at 410 nm using a Dynatech MR 600 microtiter plate reader (Dynatech Inc., Vienna, VA) interfaced with an IBM XT computer. All data were analyzed with the Immunosoft Program (Dynatech). All plasma samples were run at least in duplicate. Intra-assay and interassay coefficients of variation were 6% and 8%, respectively. No significant differences between apoA-I values in fresh plasma samples and frozen plasma samples stored at -80°C for 12 months were noted ($n = 19$; fresh = 106 ± 16 mg/dl, frozen = 105 ± 16 mg/dl, NS).

Population subjects

Subjects were participants in the 3rd examination cycle of the Framingham Offspring Study (24). Only subjects with complete lipid and apolipoprotein A-I measurements were included in this study. A total of 1,584 men (mean age: 49 ± 10 years) and 1,639 women (mean age 49 ± 10 years) were studied. Occurrence of myocardial infarction and angina pectoris was assessed as previously described (25). Hypertension was defined as a systolic blood pressure greater than 140 mm Hg or a diastolic blood pressure greater than 95 mm Hg, or use of anti-hypertensive medications. Subjects were defined as having diabetes mellitus when their plasma glucose level was greater than 140 mg/dl or when they were using insulin or hypoglycemic drugs. Body mass index values greater than 25

TABLE 1. Plasma lipid, lipoprotein, and apolipoprotein A-I levels, and characteristics of subjects participating in the Framingham Offspring Study

Variable	Men n = 1,584	Women n = 1,639	P value
TG (mg/dl)	144 ± 114	105 ± 87	0.0001
TC (mg/dl)	212 ± 39	211 ± 43	ns
VLDL-C (mg/dl)	31 ± 24	23 ± 20	0.0001
LDL-C (mg/dl)	136 ± 35	132 ± 38	0.003
HDL-C (mg/dl)	45 ± 12	57 ± 15	0.0001
ApoA-I (mg/dl)	135 ± 32	158 ± 36	0.0001
HDL-C/apoA-I	0.33 ± 0.06	0.36 ± 0.07	0.0001
TC/HDL-C	5.08 ± 1.74	3.98 ± 1.42	0.0001
Age (years)	49 ± 10	49 ± 10	ns
BMI (kg/m ²)	27.16 ± 3.82	25.35 ± 5.16	0.0001
BMI > 25.00/BMI > 30.00 (%)	72/20	43/15	0.001/0.05 ^a
Glucose (mg/dl)	98 ± 24	92 ± 20	0.0001
Diabetes (%)	4.2	2.3	0.002 ^a
Syst. blood press. (mm Hg)	125 ± 16	121 ± 18	0.0001
Diast. blood press. (mm Hg)	81 ± 9	77 ± 10	0.0001
Hypertension (%)	23.8	22.4	ns ^a
CHD (%)	6.4	3.2	0.0001 ^a
β-Blocker users (%)	10.0	6.6	0.0001 ^a
Smokers (%)	28.1	29.8	ns ^a
>One drink alcohol/week (%)	78.5	63.8	0.0001 ^a

^aChi-square test.

and 30 were selected as cutoff points for grade 1 and 2 obesity, respectively (26). Alcohol consumption and estrogen use in the participants were determined by an interview at the time of their physical examination.

Lipid analyses

After a 12–14 h overnight fast, blood was drawn into tubes containing EDTA (final concentration 1 mg/ml) and centrifuged at 2,500 rpm for 30 min at 4°C to separate plasma. Plasma aliquots were frozen and stored at –80°C for the determination of apoA-I levels. Plasma HDL cholesterol levels were measured after precipitation of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) particles with dextran sulfate-Mg²⁺ (27). To determine VLDL and LDL cholesterol concentration, plasma samples were centrifuged in a Beckman 50 Ti rotor at 39,000 rpm for 18 h at 4°C, at a density of 1.006 g/ml. The concentration of the 1.006 g/ml infranate cholesterol and plasma levels of triglycerides, total cholesterol, and HDL cholesterol were measured by automated enzymatic methods (28). VLDL and LDL cholesterol were then calculated as follows: VLDL cholesterol = total cholesterol – 1.006 g/ml infranate cholesterol; LDL cholesterol = 1.006 g/ml infranate cholesterol – HDL cholesterol.

Statistical analyses

The SAS statistical program (SAS Institute, Cary, NC) was used to perform all statistical analyses. As the distributions of plasma triglyceride and VLDL cholesterol levels were highly skewed, a logarithmic transformation

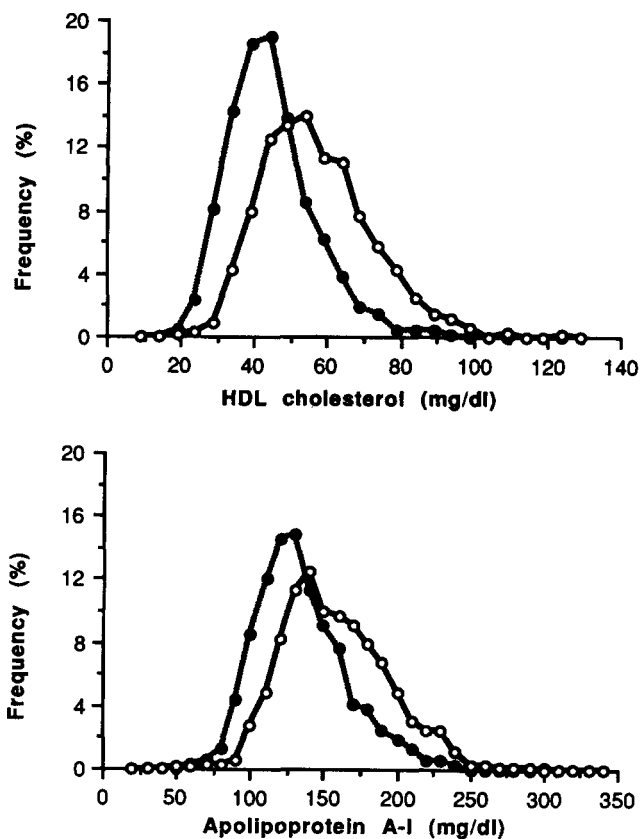


Fig. 1. Frequency distributions of plasma HDL cholesterol and apolipoprotein A-I levels in 1,584 men (dotted line, filled circles) and 1,639 women (continuous line, open circles), participating in the Framingham Offspring Study.

was applied to triglyceride and VLDL cholesterol values to approximate a normal distribution. Unpaired two-tailed *t*-tests were used to assess statistical differences between mean values. A chi-square test was applied for categorical variables. The general linear model procedure was used to adjust plasma lipid and apoA-I levels for the effect of age. Simple regression analyses were performed using the Corr procedure. Stepwise multiple regression analyses with backward elimination procedure were performed to discriminate variables affecting HDL cholesterol and apoA-I levels. Seventy postmenopausal and 19 premenopausal women taking estrogens were excluded from the analyses when so indicated in the text and tables.

RESULTS

Table 1 shows plasma levels of lipids, lipoproteins, and apoA-I in 1,584 men and 1,639 women participating in the Framingham Offspring Study. Physical characteristics and disease prevalence in these subjects are also reported (Table 1). Mean plasma levels of HDL cholesterol and apoA-I were significantly higher in women than in men (HDL: +26%, $P < 0.0001$; apoA-I: +17%, $P < 0.0001$,

respectively). Because of the greater gender-related difference in HDL cholesterol than in apoA-I, the HDL cholesterol/apoA-I ratio was also significantly higher in women than in men. Plasma triglyceride and VLDL cholesterol levels were significantly lower in women than in men (TG: -37%, $P < 0.0001$; VLDL-C: -35%, $P < 0.0001$, respectively). Body mass index and the percentage of subjects with diabetes and CHD were significantly higher in men than in women. The frequency distributions of HDL cholesterol and apoA-I levels in men and women are shown in **Fig. 1**. Clearly, as for HDL cholesterol levels, apoA-I values in women were shifted to the right, as compared to those in men.

Means and selected percentiles of plasma levels for HDL cholesterol and apoA-I are also provided by age group in these men and women (**Table 2** and **Table 3**, respectively). Plasma levels of HDL cholesterol and apoA-I were significantly higher in women taking estrogens ($n = 89$) than in those off estrogens (HDL: 62 vs. 57 mg/dl, $P < 0.01$; apoA-I: 181 vs. 157 mg/dl, $P < 0.0001$, respectively), independently from the effects of age and BMI. Therefore, means and selected percentiles for HDL cholesterol and apoA-I levels in women are also provided after exclusion of subjects taking estrogens (either alone

TABLE 2. Mean plasma levels and selected percentiles of HDL cholesterol, by age and gender, in participants in the Framingham Offspring Study

Age Group (years)	n	Mean \pm SD (mg/dl)	Percentiles (mg/dl)							
			5	10	25	50	75	90	95	
Men										
20-29 ^c	48	49 \pm 12								
30-39	272	46 \pm 12 ^a	28	33	38	44	54	63	68	
40-49	521	44 \pm 11 ^a	28	30	36	43	51	60	65	
50-59	463	45 \pm 13 ^a	29	31	36	43	50	60	64	
60-69	255	44 \pm 12 ^a	28	30	35	42	51	58	68	
70 + ^c	20	43 \pm 15 ^b								
All	1,579	45 \pm 12 ^a	28	31	37	43	51	61	67	
Women^d										
20-29 ^c	30	54 \pm 13								
	(27)	(53 \pm 13)								
30-39	312	56 \pm 13	33	39	47	55	64	75	81	
	(303)	(56 \pm 13)	(34)	(40)	(47)	(55)	(64)	(75)	(81)	
40-49	540	57 \pm 14	37	40	46	56	66	76	84	
	(521)	(57 \pm 14)	(37)	(40)	(46)	(56)	(66)	(76)	(82)	
50-59	506	57 \pm 16	35	39	46	55	67	78	86	
	(457)	(57 \pm 16)	(35)	(39)	(45)	(54)	(66)	(78)	(86)	
60-69	228	57 \pm 16	36	38	45	55	66	80	88	
	(218)	(57 \pm 16)	(35)	(38)	(45)	(54)	(64)	(79)	(88)	
70 + ^c	16	58 \pm 11								
	(16)	(58 \pm 11)								
All	1,632	57 \pm 15	35	39	46	55	66	77	84	
	(1,543)	(57 \pm 15)	(36)	(39)	(46)	(55)	(66)	(77)	(83)	

^a $P < 0.0001$; ^b $P < 0.005$, women versus men.

^cSelected percentiles for plasma HDL cholesterol levels are not reported because of the small number of subjects in these groups.

^dValues within parentheses after exclusion of women taking estrogens.

TABLE 3. Mean plasma levels and selected percentiles of apolipoprotein A-I, by age and gender, in Framingham Offsprings Study subjects

Age Group (years)	n	Mean ± SD (mg/dl)	Percentiles (mg/dl)							
			5	10	25	50	75	90	95	
Men										
20-29 ^f	48	137 ± 31								
30-39	272	136 ± 31 ^a	93	102	115	132	154	181	196	
40-49	521	134 ± 31 ^a	93	100	113	131	153	175	189	
50-59	463	135 ± 32 ^a	92	99	114	129	151	179	195	
60-69	255	137 ± 35 ^a	92	101	112	131	155	184	201	
70 + ^c	20	127 ± 35 ^b								
All	1,579	135 ± 32 ^a	92	99	114	130	153	178	196	
Women^d										
20-29 ^f	30	144 ± 31								
	(27)	(142 ± 29)								
30-39	312	151 ± 34	103	113	125	146	173	199	209	
	(303)	(151 ± 34)	(104)	(113)	(126)	(146)	(172)	(199)	(209)	
40-49	540	157 ± 36	108	116	132	152	181	206	223	
	(521)	(157 ± 35)	(108)	(116)	(131)	(152)	(180)	(204)	(219)	
50-59	506	161 ± 36	109	119	135	156	183	207	227	
	(457)	(159 ± 36)	(108)	(117)	(134)	(155)	(181)	(205)	(224)	
60-69	228	164 ± 38	109	122	136	159	187	218	234	
	(218)	(163 ± 38)	(106)	(122)	(135)	(159)	(183)	(216)	(234)	
70 + ^c	16	170 ± 41								
	(16)	(170 ± 41)								
All	1,632	158 ± 36	107	116	132	154	181	206	224	
	(1,543)	(157 ± 36)	(107)	(116)	(131)	(152)	(180)	(204)	(222)	

^a*P* < 0.0001; ^b*P* < 0.005, women versus men.

^cSelected percentiles for plasma apoA-I levels are not reported because of the small number of subjects in these groups.

^dValues within parentheses after exclusion of women taking estrogens.

or in combination with progestins) (Tables 2 and 3). There was no marked modification of plasma HDL cholesterol levels with age in men and women. No age-related changes in apoA-I levels were noted in males, while there was a relevant age-related increase in apoA-I levels in women that was independent from estrogen use. Women had significantly higher apoA-I levels than men in most age groups.

Table 4 shows mean values of plasma lipids and lipoproteins, and characteristics of subjects, by HDL cholesterol levels and gender. While plasma total cholesterol was virtually identical in subjects with high and low levels of HDL cholesterol, and LDL cholesterol levels were only marginally different between these two groups, a striking difference in triglyceride and VLDL cholesterol levels was observed between these groups, in both men and women. In addition, the HDL cholesterol/apoA-I ratio was significantly lower in subjects with low HDL cholesterol levels than in subjects with high HDL cholesterol, both in men and women. Assuming that most of apoA-I in fasting plasma is in HDL particles, this indicates a different composition of HDL particles in men and women. Interestingly, the lipoprotein profile was remarkably similar in men and women with similar HDL cholesterol levels (Table 4). One of the most striking differences between the

subjects with low HDL cholesterol levels, as compared to those with high HDL cholesterol, was observed for BMI: the percentage of subjects with BMI greater than 25 (grade 1 obesity) and greater than 30 (grade 2 obesity) was significantly higher in subjects with HDL cholesterol less than 35 mg/dl than in subjects with HDL cholesterol greater or equal to 60 mg/dl, in both males and females. In addition, the prevalence of both CHD and diabetes mellitus was several-fold higher in men and women with low HDL cholesterol levels than in subjects with high HDL cholesterol levels. Hypertension was more prevalent in the low HDL cholesterol level group only in women. Use of β -blockers was significantly greater in subjects with low HDL cholesterol than in subjects with high HDL cholesterol levels. The percentage of current cigarette smokers was significantly higher in subjects with low HDL cholesterol, in both men and women. A significantly higher percentage of female smokers than male smokers were in the low HDL category. There was a significant increase in the percentage of subjects drinking more than one drink/week (either beer, wine, or liquor) with increasing HDL cholesterol levels, particularly in men, so that 92% of the male subjects with HDL cholesterol levels \geq 60 mg/dl reported drinking more than one drink/week.

TABLE 4. Plasma lipid, lipoprotein, and apolipoprotein A-I levels, and characteristics of subjects with low, average, and high levels of HDL cholesterol

Variable	HDL, Men (mg/dl)			HDL, Women (mg/dl)		
	<35 n = 287 (18.2%)	35-59 n = 1,108 (70.1%)	≥60 n = 184 (11.7%)	<35 n = 62 (3.8%)	35-59 n = 930 (56.9%)	≥60 n = 641 (39.3%)
TG (mg/dl)	234 ± 169 ^x	130 ± 87	81 ± 53	261 ± 239 ^r	115 ± 80	75 ± 35
TC (mg/dl)	212 ± 42	212 ± 39	210 ± 34	214 ± 56	211 ± 45	211 ± 38
VLDL-C (mg/dl)	48 ± 33 ^x	29 ± 20	19 ± 12	52 ± 50 ^r	25 ± 19	17 ± 10
LDL-C (mg/dl)	133 ± 37 ^y	138 ± 34	123 ± 32	134 ± 35 ^s	138 ± 39	123 ± 35
HDL-C (mg/dl)	30 ± 4 ^x	45 ± 6	69 ± 10 ^b	30 ± 4 ^x	48 ± 7	72 ± 10
ApoA-I (mg/dl)	104 ± 17 ^x	136 ± 25	182 ± 31	106 ± 20 ^r	142 ± 25	185 ± 31
HDL-C/apoA-I	0.29 ± 0.05 ^x	0.34 ± 0.06	0.38 ± 0.06	0.30 ± 0.06 ^r	0.35 ± 0.07	0.39 ± 0.06
TC/HDL-C	7.22 ± 2.04 ^x	4.85 ± 1.14 ^d	3.11 ± 0.62 ^e	7.18 ± 2.13 ^r	4.46 ± 1.19	2.99 ± 0.62
Age (years)	51 ± 10 ^y	49 ± 10	48 ± 11	48 ± 10	49 ± 10	49 ± 10
BMI (kg/m ²)	28.61 ± 4.00 ^x	27.12 ± 3.68	25.0 ± 3.42	29.93 ± 6.00 ^r	26.23 ± 5.54	23.63 ± 3.71
BMI > 25.00/BMI > 30.00 (%)	84.4 ^x /31.3 ^x	72.1 ^y /19.6	48.4 ^z /6.4	80.7 ^r /50.9 ^r	50.7/19.5	27.0/5.7
Glucose (mg/dl)	103 ± 29 ^x	97 ± 24	94 ± 15	113 ± 40 ^r	93 ± 23	89 ± 10
Diabetes (%)	9.0 ^x	3.5	1.1	17.7 ^r	2.6	0.5
Syst blood press. (mm Hg)	127 ± 17	125 ± 15	127 ± 16	128 ± 18 ^r	122 ± 18	120 ± 18
Diast blood press. (mm Hg)	81 ± 10	81 ± 9	81 ± 10	79 ± 9 ^r	77 ± 10	76 ± 10
Hypertension (%)	27.4 ^b	23.2	22.5	45.2 ^r	23.5	18.4
CHD (%)	14.2 ^x	4.9	2.7	14.5 ^x	3.3	1.9
β-Blocker users (%)	17.7 ^x	9.1	3.3	12.9 ^x	7.8	4.2
Smokers (%)	36.3 ^{x,y}	26.4 ^r	25.1	53.2 ^x	32.4	23.6
>One drink alcohol/week (%)	67.9 ^{a,x}	79.1 ^a	91.8 ^a	43.6 ^x	58.0	74.1

^x*P* < 0.0001; ^y*P* < 0.001; and ^z*P* < 0.05, subjects with HDL-C < 35 mg/dl versus subjects with HDL-C ≥ 60 mg/dl.

^a*P* < 0.0001; ^b*P* < 0.001; and ^c*P* < 0.05, women versus men.

When we calculated the prevalence of CHD in men and women in the lower and upper quartiles for VLDL cholesterol, HDL cholesterol, and apoA-I levels, we found that the incidence of manifest CHD was significantly higher in the lower quartile than in the upper quartile for HDL cholesterol and apoA-I levels (Table 5). Based on these unadjusted incidence rates, HDL cholesterol levels had a more powerful association with CHD than apoA-I levels. The incidence of CHD was also significantly different between the lower and upper quartile for VLDL cholesterol levels.

After exclusion of women taking estrogens, plasma apoA-I levels were moderately but significantly higher in postmenopausal than in premenopausal women (161 mg/dl

TABLE 5. Prevalence of coronary heart disease (CHD) by quartile levels of VLDL cholesterol, HDL cholesterol, and apoA-I in men and women

Variable	Gender	% of CHD Subjects by Quartile			Chi ²	<i>P</i>
		1	2-3	4		
VLDL-C	M	2.7	6.7	9.4	15.3	0.0001
	F	0.8	2.8	6.4	17.9	0.0001
HDL-C	M	13.8	4.9	2.7	29.4	0.0001
	F	6.3	2.4	1.7	9.8	0.002
ApoA-I	M	10.7	5.2	4.6	10.5	0.001
	F	5.2	3.1	1.3	9.3	0.002

Women taking estrogens were excluded from the analysis. Chi² and *P* value for lower versus upper quartile.

versus 153 mg/dl, respectively; *P* < 0.0001) (Table 6). This difference was no longer significant after age-adjustment of plasma apoA-I levels (data not shown). HDL cholesterol levels were not different in the two groups of women (Table 6). Postmenopausal status was associated with significantly higher plasma triglyceride, total, and LDL cholesterol levels, higher BMI, and a higher incidence of diabetes, CHD, hypertension, and use of β-blockers.

Simple correlation analyses of plasma HDL cholesterol, apoA-I, and triglyceride levels with other plasma parameters and with age, BMI, plasma glucose, systolic and diastolic blood pressure, and alcohol consumption are reported in Table 7. As estrogens have been shown to affect some of these correlations, women taking estrogens were excluded from the analysis. Plasma HDL cholesterol and apoA-I levels were negatively associated with triglyceride and VLDL cholesterol levels in both males and females. Interestingly, the inverse correlation of triglycerides and VLDL cholesterol with apoA-I was not nearly as strong in females (*r* = -0.13 and *r* = -0.14, respectively) as it was in males (*r* = -0.26 and *r* = -0.22, respectively), and was less significant than the association of triglycerides and VLDL cholesterol with HDL cholesterol (men: *r* = -0.54 and *r* = -0.43; women: *r* = -0.47 and *r* = -0.38; respectively) (Table 7). The association of HDL cholesterol and apoA-I levels with triglyceride levels in our population is further illustrated in Fig. 2. The

TABLE 6. Plasma lipid, lipoprotein, and apolipoprotein A-I levels, and characteristics of premenopausal and postmenopausal women

Variable	Premenopausal Women n = 799	Postmenopausal Women n = 743	P
TG (mg/dl)	83 ± 51	126 ± 108	0.0001
TC (mg/dl)	193 ± 36	229 ± 42	0.0001
VLDL-C (mg/dl)	19 ± 12	28 ± 25	0.0001
LDL-C (mg/dl)	119 ± 33	145 ± 38	0.0001
HDL-C (mg/dl)	57 ± 14	57 ± 15	ns
ApoA-I (mg/dl)	153 ± 34	161 ± 37	0.0001
HDL-C/apoA-I	0.37 ± 0.07	0.35 ± 0.07	0.0001
TC/HDL-C	3.65 ± 1.24	4.36 ± 1.49	0.0001
Age (years)	42 ± 6	56 ± 7	0.0001
BMI (kg/m ²)	24.59 ± 5.04	26.17 ± 5.33	0.0001
BMI > 25.00/BMI > 30.00 (%)	34/12	51/18	0.05/ns ^a
Glucose (mg/dl)	88 ± 12	97 ± 25	0.0001
Diabetes (%)	1.1	3.6	0.001 ^a
Syst blood press. (mm Hg)	115 ± 15	128 ± 19	0.0001
Diast blood press. (mm Hg)	75 ± 9	78 ± 10	0.0001
Hypertension (%)	11.1	34.0	0.0001 ^a
CHD (%)	1.1	5.3	0.0001 ^a
β-Blocker use (%)	3.9	9.3	0.0001 ^a
Smokers (%)	30.4	29.2	ns ^a
> One drink alcohol/week (%)	64.8	62.7	ns ^a

Estrogen users were excluded from analysis.
^aChi-square test.

HDL-triglyceride relationship can be fit with the following equations:

men: HDL cholesterol = 90.56 - 9.58 × log triglycerides
women: HDL cholesterol = 110.83 - 12.02 × log triglycerides

In addition, as already suggested by the results shown in Table 3, apoA-I levels were significantly correlated with age in women, but not in men. The positive association between age and apoA-I in women was still statistically significant ($P < 0.0001$) after alcohol consumption, BMI,

cigarette smoking, plasma triglycerides, estrogen use, and blood pressure were taken into account in a multiple regression analysis (Table 8). BMI and plasma triglycerides were strong determinants of HDL cholesterol and apoA-I levels, in both men and women. As indicated in Table 8, in men, plasma triglycerides, alcohol consumption, BMI, and blood pressure were significantly associated with both HDL cholesterol and apoA-I in a stepwise multiple regression analysis. Altogether these variable explained 36% of HDL cholesterol and 12% of apoA-I variability in men. In women, age, menopausal status, estrogen use,

TABLE 7. Correlation analyses (r) of plasma apoA-I, HDL cholesterol, and triglycerides with plasma lipoproteins and other characteristics

Variable	r , Men			r , Women		
	HDL-C	ApoA-I	TG	HDL-C	ApoA-I	TG
TG	-0.54 ^a	-0.26 ^a		-0.47 ^a	-0.13 ^a	
TC	-0.03	0.10 ^a	0.45 ^a	0.03	0.16 ^a	0.48 ^a
VLDL-C	-0.43 ^a	-0.22 ^a	0.77 ^a	-0.37 ^a	-0.14 ^a	0.68 ^a
LDL-C	-0.10 ^a	-0.01	0.13 ^a	-0.18 ^a	-0.06	0.37 ^a
HDL-C		0.76 ^a	-0.54 ^a		0.76 ^a	-0.47 ^a
ApoA-I	0.76 ^a		-0.26 ^a	0.76 ^a		-0.13 ^a
Age	-0.06	0.01	0.17 ^a	0.02	0.11 ^a	0.35 ^a
BMI	-0.29 ^a	-0.17 ^a	0.29 ^a	-0.31 ^a	-0.17 ^a	0.40 ^a
Glucose	-0.12 ^a	-0.06	0.18 ^a	-0.18 ^a	-0.07	0.33 ^a
Syst blood press	-0.01	0.08	0.20 ^a	-0.07	0.07	0.34 ^a
Diast blood press	-0.04	0.06	0.20 ^a	-0.06	0.04	0.28 ^a
Alcohol	0.20 ^a	0.18 ^a	-0.04	0.19 ^a	0.19 ^a	-0.08

Women taking estrogens were excluded from the analysis.
^a $P < 0.0001$.

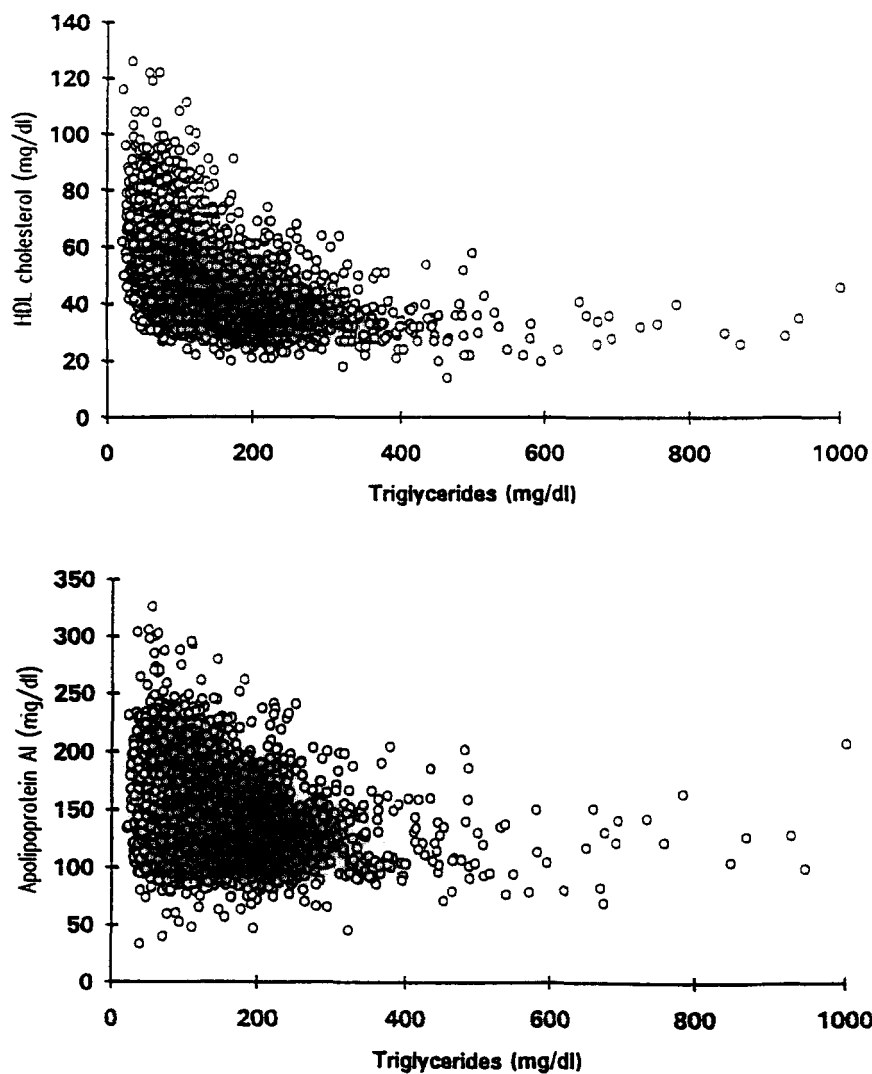


Fig. 2. Associations of HDL cholesterol and apoA-I levels with plasma triglyceride levels.

cigarette smoking, and plasma glucose levels entered into the stepwise multiple regression equation to a significant level and explained, together with triglycerides, alcohol consumption, BMI, and blood pressure, 32% and 13% of HDL cholesterol and apoA-I variability, respectively.

DISCUSSION

The Framingham Heart Study, the Multiple Risk Factor Intervention Trial, the Lipid Research Clinics Coronary Primary Prevention Trial, the Helsinki Heart Study, and other epidemiological studies have shown that low plasma HDL cholesterol levels are an independent risk factor for CHD (1-5, 29, 30). Moreover, recent NCEP guidelines indicate that elevated HDL cholesterol levels (≥ 60 mg/dl) are to be considered a protective factor in

the development of CHD (8). We have characterized male and female subjects with low (< 35 mg/dl) and elevated (≥ 60 mg/dl) plasma HDL cholesterol levels in the Framingham Offspring population. Our data clearly indicate that gender, plasma triglyceride levels, BMI, alcohol consumption, cigarette smoking, and plasma glucose levels are the parameters that best differentiate subjects with low HDL cholesterol levels from subjects with high HDL cholesterol levels in this population.

Both men and women with low HDL cholesterol have, on average, plasma triglyceride levels 3- to 3.5-fold higher than subjects with high HDL cholesterol levels. The inverse relationship between triglycerides and HDL cholesterol is well known (31). However, this relationship is not linear, as also indicated in Fig. 2. This is possibly due to the presence of a percentage of subjects with isolated hypoalphalipoproteinemia, not associated with alterations

TABLE 8. Stepwise multiple regression analysis for HDL cholesterol and apoA-I

Variable	r	Partial r ²	P Value
HDL cholesterol			
Men, r ² = 0.36			
Triglycerides	0.54	0.297	0.0001
BMI	0.17	0.030	0.0001
Alcohol consumption ^a	0.12	0.015	0.0001
Blood pressure	0.11	0.013	0.01
Women, r ² = 0.32			
Triglycerides	0.47	0.217	0.0001
Age	0.20	0.040	0.0001
Alcohol consumption ^a	0.15	0.022	0.0001
BMI	0.14	0.019	0.0001
Estrogen use ^b	0.09	0.008	0.0001
Cigarette smoking ^b	0.09	0.007	0.0001
Menopause status ^b	0.08	0.004	0.003
Blood pressure	0.04	0.003	0.03
Plasma glucose	0.04	0.002	0.05
ApoA-I			
Men, r ² = 0.12			
Triglycerides	0.26	0.067	0.0001
Alcohol consumption ^a	0.17	0.029	0.0001
Blood pressure	0.12	0.015	0.0001
BMI	0.11	0.012	0.0001
Women, r ² = 0.13			
Alcohol consumption ^a	0.18	0.034	0.0001
Age	0.16	0.026	0.0001
BMI	0.16	0.025	0.0001
Triglycerides	0.12	0.016	0.0001
Estrogen use ^b	0.12	0.015	0.0001
Cigarette smoking ^b	0.08	0.006	0.002
Blood pressure	0.07	0.005	0.003
Menopause status ^b	0.05	0.003	0.005
Plasma glucose	0.04	0.002	0.05

^aAlcohol consumption is coded as: 0 = less than 1 drink (either beer, wine, or liquor) per week, and 1 = 1 or more drinks per week.

^bEstrogen use, menopausal status, and cigarette smoking are coded as: 0 = no and 1 = yes.

in plasma triglycerides (32). The low HDL cholesterol-high triglyceride trait may be related to enhanced HDL catabolism in hypertriglyceridemia (33–35). It has been speculated that this enhanced catabolism of HDL particles is due to replacement of cholesteryl esters by triglyceride within the HDL core in the setting of hypertriglyceridemia. It is known that this exchange of lipids within the HDL core is mediated in humans by cholesteryl ester transfer protein (36). This is in agreement with our observation that subjects in the low HDL cholesterol group have a significantly lower HDL cholesterol/apoA-I ratio than subjects in the high HDL cholesterol group. Another factor that may modulate HDL phospholipid and triglyceride content within HDL is hepatic lipase. Hepatic lipase is known to be decreased by estrogens, as well as by alcohol, the only situations where both triglycerides and HDL cholesterol levels are simultaneously increased (37, 38). This effect of estrogen on both HDL cholesterol and triglyceride levels may be responsible for

the observation that the relationship between HDL cholesterol and triglycerides is less marked in women than in men. In both this study and a previous estrogen treatment study in women, estrogen use increased apoA-I levels more than HDL cholesterol levels (39). We found that, in both men and women, levels of apoA-I were correlated with both triglycerides and VLDL cholesterol levels, but the finding of a stronger inverse association between HDL cholesterol and triglycerides than between apoA-I and triglycerides indicates that apoA-I levels are less affected by hypertriglyceridemia than is HDL cholesterol.

Kinetic studies have clearly shown that, within each gender, the most important parameter regulating plasma levels of HDL cholesterol is the rate of HDL apoA-I catabolism (35, 40, 41). In subjects with low HDL cholesterol levels, an accelerated apoA-I catabolism is commonly found. Also, modification of the diet known to induce alterations in HDL cholesterol levels, such as low-fat and high-fat diets, have been shown to modulate HDL cholesterol levels by altering apoA-I transport rates (40, 41). However, there are indications that estrogens increase HDL and apoA-I levels also by increasing apoA-I production rates (42).

While grade 1 obesity is present in greater than 80% of males and females with low HDL cholesterol levels, only 50% of males and 27% of females with high HDL cholesterol have BMI values above 25. The same considerations apply when a BMI greater than 30 is considered. It is known that BMI and body composition have a marked effect on the plasma lipoprotein profile (43), and it is possible that the effect of BMI on HDL cholesterol is mediated, at least in part, through its effect on triglycerides (44). The prevalence of diabetes seems to play a significant role in hypoalphalipoproteinemia as well (44). Similarly, cigarette smoking is a parameter that differentiates subjects with low HDL cholesterol levels from those with high levels of HDL cholesterol, but it is more important in women than in men in our study. In this regard, our data are consistent with previous observations (45).

ApoA-I levels and HDL cholesterol levels were significantly higher in subjects with alcohol consumption, independent of the amount of alcohol consumed per week, than in non-drinkers, in both males and females. The positive association between alcohol and HDL cholesterol and apoA-I levels was still statistically significant after several other variables were taken into account. Although heavy alcohol consumption has been shown to increase morbidity and mortality by several causes in the general population, including myocardial infarction and stroke, several epidemiological studies have indicated that mild to moderate alcohol consumption is associated with a reduced risk of CHD (46, 47). Alcohol consumption has been shown to increase apoA-I and HDL cholesterol levels (47), and it has been hypothesized that this may be one

of the mechanisms by which mild or moderate alcohol consumption may protect against CHD. It has been postulated that the alcohol-related increase in HDL reflects the modification of the activity of the enzymes lipoprotein lipase and hepatic lipase and the cholesteryl ester transfer protein that accompany alcohol consumption (38, 48, 49).

Interestingly, when subjects were grouped on the basis of their HDL cholesterol value, a remarkably similar plasma lipoprotein profile and CHD prevalence was observed in men and women in this population, indicating that most of the age-adjusted protection from CHD in women is attributable to higher HDL cholesterol levels. Of all measured parameters, only the prevalence of diabetes and hypertension were significantly higher in women than in men in the low HDL cholesterol group.

Our data indicate that, as for HDL cholesterol, females have higher plasma levels of apoA-I than males. This has been observed in other studies with a smaller number of subjects (50). Kinetic studies have indicated that females have higher production rates for apoA-I than males and that estrogen administration increases such production rates (33, 42). Endogenous sex hormones affect both the apoA-I expression and metabolism (51). We have previously documented that HDL cholesterol levels increased by 24% and apoA-I levels by 56% when dyslipidemic postmenopausal women were given estrogen replacement (39). The fact that the gender difference in apoA-I levels persists even after the onset of menopause may be explained by the fact that progestins, which decrease HDL levels (52), are decreased as well as estrogens in postmenopausal women. Alternatively, other age-related mechanisms may be associated with the increase in apoA-I with aging in women. When apoA-I plasma levels were age-adjusted, the difference in apoA-I between premenopausal and postmenopausal women was no longer significant, as also observed in a previous study (53). These data suggest that the increased rate of CHD in postmenopausal women, as compared to premenopausal women, is more related to changes in LDL cholesterol than to changes in HDL cholesterol levels.

There is controversy as to whether the measurement of plasma apoA-I levels provides information about CHD risk in addition to that provided by HDL cholesterol levels in clinical studies (17-20). Our data suggest that both HDL cholesterol and apoA-I levels are good indicators of CHD in men and women. However, these findings need to be confirmed in large prospective studies. The measurement of apoA-I levels in the population presented here will provide such information in the near future.

The triglyceride-HDL cholesterol-BMI interrelationship supports the concept that normalization of body weight, through dietary modification and physical exercise, will improve plasma levels of both triglyceride and HDL cholesterol, as it has been previously documented

(54). Patients with low HDL cholesterol levels should be directed towards optimizing body weight and adequate treatment of other known CHD risk factors (elevated LDL cholesterol, cigarette smoking, diabetes, hypertension) (55). ■■

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