Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study

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Abstract Plasma low density lipoprotein (LDL) cholesterol, non-high density lipoprotein (HDL) cholesterol, and apolipoprotein (apo) B, the major protein constituent of LDL, were measured in 1,533 men (mean age 49 ± 10 years) and 1,597 women (mean age 49 ± 10 years) participating in the 3*rd* examination cycle of the Framingham Offspring Study. Mean plasma levels of LDL cholesterol and apoB were higher in men than in women (136 versus 132 mg/dl, $P < 0.0001$; and 109 versus 95 mg/dl, $P < 0.0001$, respectively). Increased age was associated with higher plasma LDL cholesterol and apoB levels, especially in women. After adjustment for age and body mass index, LDL cholesterol and apoB levels were still significantly higher in postmenopausal than in premenopausal women, indicating a hormonal effect on LDL metabolism. The associations between coronary heart disease (CHD) and LDL cholesterol, non-HDL cholesterol, apoB, and other plasma lipid and lipoprotein parameters were examined by dividing participants in four groups, based on approximate quartiles for these parameters. Elevated LDL cholesterol levels were not significantly associated with CHD in men, but were in women. This result, at variance with that of several longitudinal studies, is likely due to the cross-sectional design of our analysis. Elevated non-HDL cholesterol and apoB levels were significantly associated with the presence of CHD, in both males and females. A plasma apoB value ≥ 125 mg/dl may be associated with an increased risk for CHD. Low plasma levels of HDL cholesterol were also significantly associated with CHD. Plasma triglyceride levels, age and body mass index were strong determinants of LDL cholesterol, non-HDL cholesterol, and apoB levels in men and women. In women, postmenopausal status and elevated blood pressure were also significantly associated with elevated levels of these parameters.—**Schaefer, E. J., S. Lamon-Fava, S. D. Cohn, M. M. Schaefer, J. M. Ordovas, W. P. Castelli, and P. W. F. Wilson.** Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. *J. Lipid Res.* 1994. **35:** 779–792.

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The Framingham Heart Study has been instrumental in the characterization of the risk factors for coronary heart disease (CHD). Findings in this population as well as in others have clearly indicated an association between plasma total and low density lipoprotein (LDL) cholesterol and CHD (1–5). It has been shown that most of the increased CHD risk conferred by elevated levels of total cholesterol is due to elevations of the LDL cholesterol fraction (2, 4, 6). Based on results from intervention and epidemiological studies, the National Cholesterol Education Program (NCEP) Expert Panel has provided guidelines for the classification of LDL cholesterol elevations (7). According to these guidelines, an LDL cholesterol level below 130 mg/dl is considered desirable, while an LDL cholesterol level greater than or equal to 160 mg/dl is defined as a high risk factor for CHD, in both men and women.

The Framingham Offspring Study was originally designed to study risk factors for both the development of CHD in individuals and the familial clustering of CHD in the adult offspring of the participants in the Framingham Heart Study (8). The Offspring study offers the opportunity to test new hypotheses and use new and more sophisticated techniques in characterizing the factors associated with the development of premature CHD. Some studies, but not all, have suggested that the measurement of apoB in plasma may provide more information on CHD risk than LDL cholesterol alone (9–12).

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; CHD, coronary heart disease; BMI, body mass index; TC, total cholesterol; VLDL, very low density lipoproteins.

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Previous studies have shown a significant impact of age on plasma LDL cholesterol levels (13). Plasma LDL cholesterol levels increase progressively from young adulthood to approximately age 60 in men, and to age 70 in women, and then decrease. This decrease in LDL cholesterol levels may be partly, but not entirely, due to selective survival of individuals with lower cholesterol levels. In fact, results from the Framingham Heart Study clearly indicate that total cholesterol levels decrease after age 60 in individuals followed longitudinally (14). In addition, both gender- and menopause-associated differences in plasma LDL cholesterol levels have been observed, and have been attributed to the effect of sex hormones on LDL metabolism (15). Apolipoprotein (apo) B is the protein constituent of LDL and mediates the uptake of LDL particles by both liver and extrahepatic tissues by a specific interaction with the LDL receptor (16, 17). Very little information is at present available on the effects of age, gender, and menopause on apoB levels in large populations. As there are indications that subjects with increased apoB levels, but normal LDL cholesterol levels, may have an increased risk of CHD, it has been suggested that apoB levels should be measured, in addition to LDL cholesterol, in assessing CHD risk (18, 19).

The purpose of this study was to provide normal ranges for apoB levels and to evaluate the effect of age, gender, and menopausal status on plasma levels of LDL cholesterol and apoB in the Framingham Offspring population. We have attempted to define the most important factors responsible for LDL cholesterol and apoB variability in this population. In addition, we have determined the relationship between these plasma parameters and CHD in a cross-sectional analysis. These data will be used to assess the relative importance of apoB versus LDL cholesterol as CHD risk factors prospectively.

MATERIALS AND METHODS

Population

Subjects were participants in the 3rd examination cycle of the Framingham Offspring Study (8). All subjects were Caucasian. Only subjects with complete plasma lipid measurements were included in the study. A total of 1,533 men (mean age: 49 ± 10 years) and 1,597 women (mean age: 49 ± 10 years) were studied. Occurrence of myocardial infarction and/or angina pectoris in these subjects was assessed as previously described (20). Subjects were defined as having hypertension when their systolic blood pressure was greater than 140 mm Hg, their diastolic blood pressure was greater than 95 mm Hg, or they were being treated for hypertension. Diabetes mellitus was defined as having fasting plasma glucose levels greater than 140 mg/dl or use of oral hypoglycemic medications or insulin.

Lipid and lipoprotein analyses

Blood from each subject was drawn, after a 12–14 h overnight fast, into tubes containing ethylenediaminetetraacetic acid (EDTA) at a final concentration of 1 mg/ml. Blood was centrifuged at 2,500 rpm for 30 min at 4°C to separate plasma. Plasma high density lipoprotein (HDL) cholesterol was measured after precipitation of apoB-containing lipoproteins with dextran sulfate–Mg^{2+} (21). Plasma total cholesterol, HDL cholesterol, and triglycerides were measured by automated enzymatic techniques (22). For the measurement of plasma very low density lipoprotein (VLDL) and LDL cholesterol, plasma was 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. Plasma VLDL and LDL cholesterol values were then obtained as follows: VLDL cholesterol = plasma total cholesterol −1.006 g/ml infranate cholesterol; plasma LDL cholesterol = 1.006 g/ml infranate cholesterol − HDL cholesterol. In clinical practice, serum values for total cholesterol are used more frequently than plasma values, and LDL cholesterol values are calculated by the Friedewald formula (23). Values for serum cholesterol are on average 3% higher than plasma values (24). Accordingly, we have calculated total serum cholesterol values as follows: serum total cholesterol = plasma total cholesterol × 1.03. LDL was then calculated with the Friedewald formula as follows: serum LDL cholesterol = serum total cholesterol − (triglycerides/5 + HDL cholesterol). Our laboratory participates in the Centers for Diseases Control (CDC) lipid standardization program, and serves as one of the ten network laboratories.

LDL size was determined by gradient gel electrophoresis on 2–16% non-denaturing polyacrylamide gels (Pharmacia, Piscataway, NJ) as previously described (25). Seven separate LDL bands can be identified by this method in the general population (LDL1 being the largest and LDL7 the smallest) (25, 26). As the majority of individuals have, in addition to one major band, one or two minor LDL bands, an LDL particle score taking into account the relative areas under all the major and minor LDL bands was calculated for each individual as previously indicated (27).

Apolipoprotein B assay

ApoB was measured by a noncompetitive enzyme-linked immunosorbent assay (ELISA) using affinity-purified polyclonal anti-apoB antibodies. An accurate description of the anti-apoB antibody preparation, the ELISA procedure, and the calibration of the assay is provided by Ordovas et al. (28). Briefly, microtiter plates (Nunc Immunoplate Inc., Nunc, Denmark) coated with immunopurified polyclonal anti-apoB antibody were loaded with plasma samples diluted 1:3000. After an overnight incubation at room temperature plates were extensively washed and then incubated for 5 h with an alkaline

phosphatase-conjugated anti-apoB antibody. Plates were then washed and the final apoB concentration was determined by a colorimetric reaction, using the alkaline phosphatase substrate disodium *p*-nitrophenylphosphate as reagent (Sigma, St. Louis, MO). Plates were read on a microtiter plate reader (MR 600, Dynatech Inc., Vienna, VA) interfaced with an IBM XT personal computer. Our assay was standardized with LDL-apoB-100 (d 1.030–1.050 g/ml; this LDL preparation contained only apoB, as assessed by SDS gel electrophoresis), whose apoB concentration had been determined by both amino acid quantitation and Lowry methods (29). Our assay accurately measures both VLDL-apoB-100 and LDL-apoB-100. As apoB-48 is identical to the amino-terminal portion of apoB-100, no assay is presently available that can measure only apoB-48. However, due to the rapid clearance of chylomicrons in the plasma (30), in the majority of individuals the amount of apoB-48 in fasting plasma is negligible and it can be generally assumed that the measured plasma apoB represents apoB-100. In fact, the apoB-100/apoB-48 ratio in triglyceride-rich lipoproteins (d < 1.006 g/ml) after an overnight fast is approximately 100:1 (31).

Statistical analyses

The SAS statistical program (SAS Institute, Cary, NC) was used to perform all statistical analyses. As the distribution of triglyceride and VLDL cholesterol levels was markedly skewed, a logarithmic transformation was applied to these parameters in order to approximate a normal distribution. Unpaired *t*-tests were used to assess statistical differences between mean values. Simple correlation analyses were performed using the CORR procedure. Differences in disease prevalence were assessed by chi-square analysis. Adjustment for age and BMI was performed by analysis of covariance. Stepwise multiple regression analyses with backward elimination procedure were performed to discriminate variable affecting plasma LDL cholesterol, non-HDL cholesterol, and apoB levels. In this analysis, variables that contributed the least to the fit of the model were progressively removed.

RESULTS

Mean age, body mass index (BMI), and other characteristics in 1,533 men and 1,597 women participating in the Framingham Offspring Study are shown in **Table 1.** Table 1 also provides mean plasma levels of triglycerides, total cholesterol, VLDL cholesterol, LDL cholesterol, non-HDL cholesterol, HDL cholesterol, and apoB, as well as total cholesterol to HDL cholesterol ratio and LDL particle score in these subjects. Plasma levels of total cholesterol were not different in men and women. However, LDL cholesterol and non-HDL cholesterol levels were significantly higher in men than in women (+3%, $P < 0.0001$; and +8.4%, $P < 0.0001$, respectively), and HDL cholesterol levels were significantly higher in women than in men (+26.6%, $P < 0.0001$). Plasma apoB concentrations were significantly higher in men than in women (+14.7%, $P < 0.0001$). A different composition of LDL particles in these two groups, as indi-

TABLE 1. Characteristics, disease prevalence, and plasma lipid, lipoprotein, and apolipoprotein B levels in men and women participating in the Framingham Offspring Study

Variable	Men n = 1,533	Women n = 1,597	*P*
Age (years)	49 ± 10	49 ± 10	NS
BMI (kg/m²)[a]	27.16 ± 3.87	25.36 ± 5.19	0.0001
BMI > 30.00 (%)	18.1	13.8	0.01[a]
Diabetes (%)	3.8	2.0	0.002[a]
Hypertension (%)	23.4	22.2	NS[a]
CHD (%)	6.5	3.2	0.0001[a]
β-Blocker users (%)	10.1	6.6	0.001[a]
Cigarette smokers (%)	28.5	30.1	NS[a]
>One drink alcohol/week (%)	78.4	63.5	0.0001[a]
Triglycerides (mg/dl)	143 ± 113	105 ± 87	0.0001
Total cholesterol (mg/dl)	212 ± 39	211 ± 43	NS
VLDL cholesterol (mg/dl)	31 ± 24	23 ± 19	0.0001
LDL cholesterol (mg/dl)	136 ± 35	132 ± 38	0.0001
Non-HDL cholesterol (mg/dl)	167 ± 41	154 ± 45	0.0001
HDL cholesterol (mg/dl)	45 ± 12	57 ± 15	0.0001
TC/HDL cholesterol	5.07 ± 1.74	3.99 ± 1.43	0.0001
Apolipoprotein B (mg/dl)	109 ± 34	95 ± 31	0.0001
LDL particle score[b]	3.42 ± 1.18	2.52 ± 1.06	0.0001

Values are expressed as mean ± SD.
[a]Chi-square test.
[b]LDL particle score was measured in 1,419 men and 1,466 women.

cated by higher mean LDL particle score in men than in women (a higher score reflects a smaller LDL particle size), was observed as well (Table 1).

The relative frequency distribution of LDL cholesterol and apoB levels in men and women is shown in **Fig. 1:** the distribution curve of both plasma parameters was shifted towards higher values in men, as compared to women. LDL cholesterol levels ranged from 36 to 315 mg/dl in men and from 47 to 316 mg/dl in women. In men apoB levels ranged between 28 and 365 mg/dl and in women between 19 and 279 mg/dl.

Plasma levels of LDL cholesterol and apoB were also analyzed by age and gender (**Table 2** and **Table 3**). Mean plasma levels of LDL cholesterol increased with age in both men and women, but more markedly in the latter group. This resulted in significantly higher LDL cholesterol levels in women than in men after age 60. Table 2 also reports LDL cholesterol levels calculated from serum total cholesterol using the Friedewald formula. Approximately 24% of male and 22% of female participants had LDL cholesterol levels ≥ 160 mg/dl when assessed in plasma after ultracentrifugation. These percentages were even higher, 33% in men and 28% in women, when serum LDL cholesterol levels were calculated by the Friedewald formula after converting plasma total cholesterol values to serum values. The percentage of subjects with elevated LDL cholesterol levels (LDL cholesterol ≥ 160 mg/dl) increased progressively with age in both groups, but more markedly in women after menopause. ApoB plasma levels also increased significantly with age in both men and women, but in contrast to LDL cholesterol, plasma apoB levels were significantly lower in women than in men in most age groups (Table 3).

Plasma (and serum) total cholesterol levels and plasma triglyceride levels, by age and gender, are shown in **Table 4** and **Table 5.** These parameters also increased significantly with age. LDL particle score, reflecting the size of LDL particles, was also measured in 1,419 men and 1,466 women. An age-related increase in LDL particle score was observed in both men and women (**Fig. 2**).

As the NCEP Expert Panel have selected values of 130 mg/dl and 160 mg/dl as LDL cholesterol level cutoff points for low and high risk of CHD, respectively, we have determined the plasma lipoprotein profile in men and women with LDL cholesterol levels less than 130 mg/dl, between 130 and 159 mg/dl, and greater than or equal to 160 mg/dl (**Table 6**). Both men and women with LDL cholesterol ≥ 160 mg/dl were on average older, had a higher BMI, and a higher prevalence of hypertension than subjects with LDL cholesterol levels less than 130 mg/dl. In addition, women with high LDL cholesterol levels were more likely to have CHD, use β-blockers, and be cigarette smokers than women with low LDL cholesterol levels. As expected, plasma lipid profiles in both males and females with elevated LDL cholesterol levels were significantly different from those of subjects with low LDL cholesterol levels (Table 6).

We determined the difference in the prevalence of CHD between subjects in the lower and upper quartile for LDL cholesterol, non-HDL cholesterol, apoB, and other plasma lipid parameters (**Table 7**). Elevated levels of LDL cholesterol, both measured and calculated with the Friedewald formula, were not associated with CHD status in males in this population, as also reported in Table 6. Similar results were also observed when subjects using β-blockers were excluded from the analysis (data not shown). In contrast, women with high risk LDL cholesterol levels had a significantly higher prevalence of CHD than women with optimal LDL cholesterol levels. However, when non-HDL cholesterol levels were evaluated, the prevalence of CHD in subjects in the upper quartile for non-HDL cholesterol was significantly higher than in subjects in the lower quartile in both men and women. The prevalence of CHD was also significantly higher in the upper quartile than in the lower quartile for apoB levels in both men and women. Our results indicate

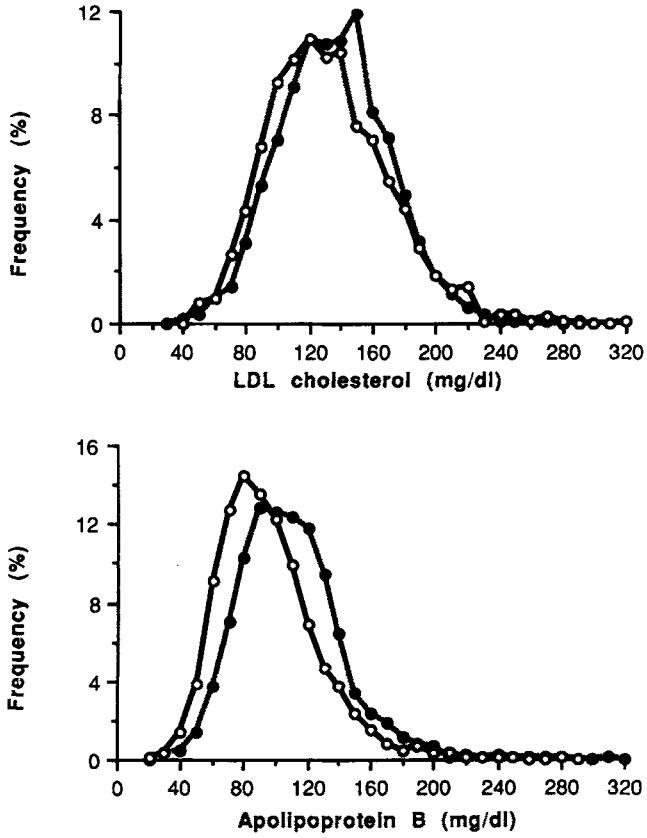

Fig. 1. Relative frequency distributions of plasma LDL cholesterol and apoB levels in 1,533 men (dotted line, filled circles) and 1,597 women (continuous line, open circles) participating in the third examination cycle of the Framingham Offspring Study.

TABLE 2. Means and selected percentiles of LDL cholesterol levels, and percentage of subjects with LDL cholesterol levels ≥ 160 mg/dl, by age and gender

Age Group (years)	n	Mean ± SD (mg/dl)	LDL-C ≥ 160 (%)	Percentiles (mg/dl)						
				5	10	25	50	75	90	95
Men										
20–29[c]	50	115 ± 31	8.0							
		(121 ± 30)	(10.0)							
30–39	261	126 ± 33[a]	16.6	76	84	101	124	150	168	180
		(135 ± 36)[a]	(25.7)	(80)	(89)	(106)	(132)	(163)	(184)	(197)
40–49	505	134 ± 34[a]	22.9	78	92	110	134	156	175	190
		(143 ± 37)[a]	(32.8)	(82)	(96)	(121)	(143)	(168)	(189)	(204)
50–59	450	143 ± 32	28.7	94	102	120	144	163	184	197
		(153 ± 35)	(39.3)	(101)	(110)	(128)	(153)	(172)	(196)	(210)
60–69	248	140 ± 37[b]	27.8	86	100	116	137	165	184	201
		(151 ± 39)[b]	(37.0)	(96)	(111)	(126)	(147)	(175)	(201)	(214)
70 +[c]	19	131 ± 33[b]	15.7							
		(137 ± 31)[b]	(21.0)							
All	1,533	136 ± 35[a]	23.8	82	93	111	135	158	178	192
		(145 ± 37)[a]	(33.4)	(87)	(98)	(121)	(143)	(169)	(192)	(206)
Women										
20–29[c]	30	114 ± 30	6.7							
		(119 ± 32)	(10.0)							
30–39	305	113 ± 33	9.2	65	73	91	110	131	157	174
		(120 ± 33)	(11.5)	(73)	(82)	(95)	(117)	(138)	(164)	(179)
40–49	529	124 ± 33	14.6	76	84	101	122	144	167	179
		(131 ± 34)	(17.6)	(82)	(91)	(108)	(130)	(151)	(176)	(187)
50–59	496	144 ± 39	31.3	90	97	116	140	168	199	211
		(153 ± 41)	(40.9)	(95)	(105)	(123)	(150)	(177)	(211)	(224)
60–69	221	147 ± 36	37.1	92	106	122	145	169	190	209
		(159 ± 38)	(46.1)	(99)	(112)	(134)	(156)	(181)	(205)	(228)
70 +[c]	16	164 ± 27	56.3							
		(178 ± 31)	(75.0)							
All	1,597	132 ± 38	22.2	76	86	105	129	155	180	199
		(140 ± 40)	(28.1)	(83)	(94)	(111)	(137)	(164)	(191)	(212)

Plasma LDL cholesterol measured by ultracentrifugation, as described in Materials and Methods. Serum LDL cholesterol, calculated by the Friedewald formula within parentheses.

[a] $P < 0.0001$, men vs. women.

[b] $P < 0.05$, men vs. women.

[c] Selected percentiles for plasma LDL cholesterol levels are not reported because of the small number of subjects in these groups.

TABLE 3. Means and selected percentiles of plasma apo B levels, by age and gender

Age Group (years)	n	Mean ± SD (mg/dl)	Percentiles (mg/dl)						
			5	10	25	50	75	90	95
Men									
20–29[c]	50	86 ± 26							
30–39	261	100 ± 31[a]	58	65	79	97	118	137	148
40–49	505	109 ± 33[a]	65	71	87	106	128	148	168
50–59	450	115 ± 34[a]	71	79	93	111	131	153	170
60–69	248	115 ± 36[b]	67	75	90	112	133	160	181
70 +[c]	19	105 ± 36							
All	1,533	109 ± 34[a]	63	71	87	106	127	149	170
Women									
20–29[c]	30	78 ± 21							
30–39	305	81 ± 27	47	52	63	76	95	115	123
40–49	529	90 ± 27	53	59	72	88	104	125	143
50–59	496	104 ± 33	60	67	81	99	121	145	165
60–69	221	108 ± 33	58	73	86	105	126	150	160
70 +[c]	16	114 ± 36							
All	1,597	95 ± 31	54	59	73	91	112	137	153

[a] $P < 0.001$, men vs. women.

[b] $P < 0.05$, men vs. women.

[c] Selected percentiles for plasma apoB levels are not reported because of the small number of subjects in these groups.

TABLE 4. Means and selected percentiles of total cholesterol levels, and percentage of subjects with total cholesterol levels ≥240 mg/dl, by age and gender

Age Group (years)	n	Mean ± SD (mg/dl)	TC ≥ 240 (%)	Percentiles (mg/dl)						
				5	10	25	50	75	90	95
Men										
20–29[c]	50	183 ± 32	6.0							
		(188 ± 33)	(8.0)							
30–39	261	199 ± 37[a]	14.9	143	153	173	198	225	251	272
		(205 ± 39)[a]	(19.5)	(147)	(157)	(178)	(203)	(232)	(259)	(280)
40–49	505	210 ± 37[a]	20.0	153	164	185	210	233	257	268
		(217 ± 38)[a]	(24.3)	(157)	(169)	(191)	(216)	(240)	(265)	(276)
50–59	450	219 ± 37[b]	26.2	164	174	192	217	240	268	281
		(226 ± 38)[b]	(31.3)	(169)	(179)	(198)	(224)	(247)	(276)	(289)
60–69	248	221 ± 42[b]	27.4	165	174	192	218	241	270	290
		(227 ± 43)[b]	(35.9)	(170)	(179)	(198)	(225)	(248)	(278)	(299)
70 +[c]	19	204 ± 35[a]	15.8							
		(210 ± 36)[a]	(15.8)							
All	1,533	212 ± 39[a]	21.6	153	165	185	210	235	262	277
		(218 ± 40)[a]	(26.8)	(158)	(170)	(191)	(216)	(242)	(270)	(285)
Women										
20–29[c]	30	181 ± 31	3.3							
		(187 ± 32)	(10.0)							
30–39	305	185 ± 35	7.5	139	145	160	183	204	232	246
		(191 ± 36)	(9.8)	(143)	(149)	(165)	(188)	(210)	(239)	(253)
40–49	529	201 ± 35	13.2	148	156	175	200	223	245	259
		(207 ± 36)	(17.2)	(152)	(161)	(180)	(206)	(230)	(252)	(267)
50–59	496	229 ± 44	36.7	167	180	198	225	253	282	299
		(235 ± 45)	(41.9)	(172)	(185)	(203)	(232)	(261)	(290)	(308)
60–69	221	234 ± 39	40.3	178	187	205	231	258	287	303
		(241 ± 41)	(47.5)	(183)	(193)	(211)	(238)	(266)	(296)	(312)
70 +[c]	*16*	*255* ± 32	62.5							
		(263 ± 32)	(68.7)							
All	1,597	211 ± 43	23.5	148	158	181	208	237	266	287
		(218 ± 44)	(28.1)	(152)	(163)	(186)	(214)	(244)	(274)	(296)

Calculated serum total cholesterol values (= plasma total cholesterol × 1.03) is within parentheses.
[a] $P < 0.0001$, men vs. women.
[b] $P < 0.001$, men vs. women.
[c] Selected percentiles for total cholesterol levels are not reported because of the small number of subjects in these groups.

TABLE 5. Mean concentrations and selected percentiles of plasma triglycerides, by age and gender

Age Group (years)	n	Mean ± SD (mg/dl)	Percentiles (mg/dl)						
			5	10	25	50	75	90	95
Men									
20–29[c]	50	90 ± 37[b]							
30–39	261	122 ± 89[a]	40	51	64	97	161	220	281
40–49	505	144 ± 131[a]	44	52	71	111	170	268	342
50–59	450	144 ± 87[b]	50	60	86	120	182	248	294
60–69	248	167 ± 142[b]	57	63	89	133	201	291	401
70 +[c]	19	153 ± 93							
All	1,533	143 ± 113[a]	46	55	75	112	173	252	324
Women									
20–29[c]	30	67 ± 28							
30–39	305	77 ± 45	37	41	50	66	90	122	151
40–49	529	91 ± 56	39	44	55	74	110	164	212
50–59	496	124 ± 112	45	52	69	99	146	217	245
60–69	221	135 ± 114	54	63	80	114	162	215	269
70 +[c]	16	134 ± 63							
All	1,597	105 ± 87	41	46	59	83	124	182	226

[a] $P < 0.0001$, men vs. women.
[b] $P < 0.05$, men vs. women.
[c] Selected percentiles for plasma triglyceride levels are not reported because of the small number of subjects in these groups.

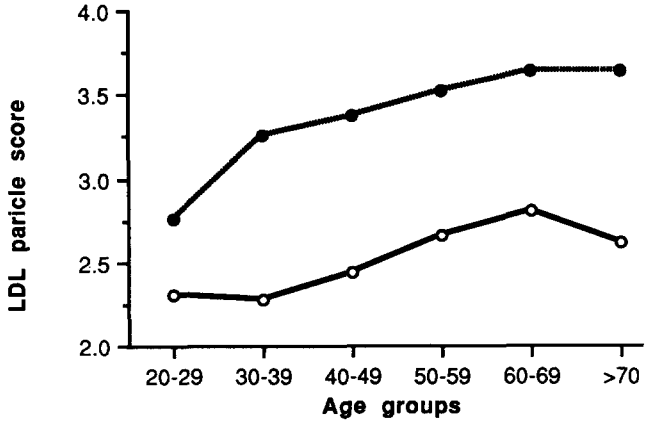

Fig. 2. LDL particle score in men (dotted line, filled circles) and women (continuous line, open circles), by age group.

that, in men and women, both the total cholesterol to HDL cholesterol ratio and the apoB to apoA-I ratio best discriminate subjects with CHD from subjects free of symptomatic cardiovascular disease (Table 7). Furthermore, when a value of 5 is used as cutoff point for total cholesterol to HDL cholesterol ratio, in both males and females a significantly higher prevalence of CHD is observed in subjects with TC/HDL greater than 5 than in individuals with TC/HDL less than 5 (men: 9.3% versus 4.2%, chi^2 = 16.3, $P < 0.0001$; women: 8.7% versus 1.9%, chi^2 = 37.5, $P < 0.0001$).

Plasma lipid, lipoprotein, and apoB levels in premenopausal and postmenopausal women are reported in **Table 8.** In postmenopausal women, plasma levels of total cholesterol were significantly higher than in premenopausal women. This was for the most part due to higher LDL cholesterol and VLDL cholesterol levels in postmenopausal women, resulting in higher non-HDL cholesterol levels, as no significant differences in HDL cholesterol were observed after menopause. Plasma apoB levels were also significantly higher and LDL particles were significantly smaller in postmenopausal than in premenopausal women. After adjustment of plasma lipid parameters for age and BMI, similar results were obtained, with the exception of LDL particle score. In fact, when LDL particle score was adjusted for plasma triglyceride levels, age, and BMI no statistical difference was observed between premenopausal and postmenopausal women (data not shown).

As indicated in **Table 9,** LDL cholesterol and apoB levels were positively associated with age, BMI, blood pressure, and most plasma lipid levels in both men and women, with the exception of HDL cholesterol, which was inversely associated with these two parameters. Plasma triglyceride levels, age, and BMI were important determinants of plasma LDL cholesterol, non-HDL cholesterol, and apoB levels, in both men and women, as indicated by multiple regression analyses (**Table 10**). However, plasma triglyceride levels, age, BMI, smoking, and blood pressure could explain a much greater portion

TABLE 6. Characteristics, disease prevalence, and plasma levels of lipids, lipoproteins, and apoB in men and women participating in the Framingham Offspring Study, by LDL cholesterol levels

Variable	LDL-C, Men (mg/dl)			LDL-C, Women (mg/dl)		
	<130 n = 669 (43.6%)	130–159 n = 499 (32.6%)	≥160 n = 365 (23.8%)	<130 n = 809 (50.6%)	130–159 n = 434 (27.2%)	≥160 n = 354 (22.2%)
Age (years)	48 ± 11^{c,x}	50 ± 10	51 ± 9^{a}	46 ± 9^{x}	50 ± 9	54 ± 9
BMI (kg/m^2)	26.70 ± 4.03^{a,y}	27.38 ± 3.86^{a}	27.68 ± 3.47^{c}	24.59 ± 5.11^{x}	25.75 ± 4.93	26.65 ± 5.35
BMI > 25 (%)	57.7^{a}	66.9^{a}	71.0	30.2	42.2	52.0
BMI > 30 (%)	17.6^{a}	17.6	19.7	11.3	14.3	18.9
Diabetes (%)	3.9^{c}	3.6	3.9	1.6	1.6	3.1
Hypertension (%)	21.2^{c,z}	22.8	28.2^{c}	15.6^{x}	21.9	37.6
CHD (%)	6.0^{a}	6.6^{c}	7.4	1.4^{x}	3.7	6.8
β-Blockers (%)	10.2^{a}	9.2	11.2	4.0^{x}	7.6	11.3
Smokers (%)	25.1	30.7	31.8	27.7^{z}	29.3	36.7
> One drink alcohol/week (%)	78.0^{a}	78.2^{a}	79.5^{a}	65.6	62.1	60.3
TG (mg/dl)	143 ± 146^{a,y}	136 ± 81^{a}	150 ± 75^{c}	92 ± 95^{x}	105 ± 55	136 ± 92
TC (mg/dl)	183 ± 29^{x}	218 ± 20^{c}	255 ± 28^{b}	183 ± 28^{x}	221 ± 20	264 ± 36
VLDL-C (mg/dl)	31 ± 29^{a}	29 ± 18^{a}	33 ± 18^{c}	20 ± 20^{x}	23 ± 14	30 ± 21
LDL-C (mg/dl)	105 ± 18^{c,x}	144 ± 8	181 ± 21^{c}	102 ± 18^{x}	143 ± 9	186 ± 24
NonHDL-C (mg/dl)	137 ± 32^{a,x}	174 ± 21^{a}	212 ± 29	124 ± 29^{x}	165 ± 18	211 ± 37
HDL-C (mg/dl)	46 ± 14^{a,y}	44 ± 11^{a}	44 ± 10^{a}	59 ± 15^{x}	56 ± 15	53 ± 13
TC/HDL-C	4.33 ± 1.62^{a,x}	5.25 ± 1.38^{a}	6.15 ± 1.76^{a}	3.29 ± 1.07^{x}	4.17 ± 1.02	5.33 ± 1.53
ApoB (mg/dl)	90 ± 26^{a,x}	114 ± 24^{a}	138 ± 36^{b}	76 ± 21^{x}	103 ± 19	129 ± 31
LDL particle score	3.34 ± 1.33^{a}	3.44 ± 1.07^{a}	3.55 ± 1.00^{a}	2.37 ± 1.05^{x}	2.58 ± 1.01	2.83 ± 1.06

^{a} < 0.0001; $^{b}P < 0.001$; $^{c}P < 0.05$; males versus females.
$^{x}P < 0.0001$; $^{y}P < 0.001$; $^{z}P < 0.05$; subjects with LDL cholesterol <130 mg/dl versus subjects with LDL cholesterol ≥160 mg/dl.

TABLE 7. Selected percentiles and prevalence of CHD by different plasma lipid and apolipoprotein parameters

	Selected Percentiles							% of CHD Subjects				
Gender	5	10	25	50	75	90	95	<25	25–75	>75	Chi²	*P*
LDL cholesterol (after ultracentrifugation of plasma)												
M	82	93	111	135	158	178	192	5.1	6.9	7.2	1.5	ns
F	76	86	105	129	155	180	199	0.8	3.0	6.1	16.9	0.0001
LDL cholesterol (calculated from serum values)												
M	87	98	121	143	169	192	206	6.0	6.1	7.3	0.1	ns
F	83	94	111	137	164	191	212	1.5	2.9	5.5	9.5	0.005
Non-HDL cholesterol												
M	102	116	139	165	193	220	234	3.8	7.3	8.4	6.9	0.01
F	92	101	122	151	182	213	233	1.0	2.3	7.2	19.1	0.0001
ApoB												
M	63	71	87	106	127	149	170	3.7	6.6	9.1	9.6	0.002
F	54	59	73	91	112	137	153	0.3	2.5	7.6	28.0	0.0001
HDL cholesterol												
M	28	31	37	43	51	61	67	13.8	4.9	2.7	29.4	0.0001
F	36	39	46	55	66	77	84	6.2	2.4	2.1	7.9	0.005
TC/HDL-C												
M	2.79	3.12	3.89	4.81	5.98	7.16	7.97	3.4	5.2	12.3	21.2	0.0001
F	2.31	2.53	2.96	3.68	4.68	5.78	6.67	1.8	1.9	7.3	14.3	0.0001
LDL-C/HDL-C												
M	1.49	1.78	2.41	3.15	3.97	4.82	5.34	4.4	5.0	11.5	13.2	0.0001
F	1.13	1.32	1.69	2.32	3.11	3.97	4.56	2.3	1.9	7.0	10.3	0.001
ApoB/ApoA-I												
M	0.40	0.48	0.61	0.81	1.03	1.29	1.49	3.6	5.6	11.3	16.5	0.0001
F	0.30	0.34	0.44	0.58	0.77	1.01	1.16	1.5	2.0	7.3	16.5	0.0001
LDL particle score												
M	1.52	2.00	2.85	3.23	4.28	5.00	5.28	3.8	6.4	10.2	13.1	0.0001
F	1.30	1.37	1.66	2.36	3.15	4.00	4.65	2.2	2.3	6.3	9.3	0.002

Chi² value compares the upper and lower quartiles.

TABLE 8. Characteristics, disease prevalence, and plasma lipid, lipoprotein and apoB levels in premenopausal and postmenopausal women participating in the Framingham Offspring Study

	Menopause		
Variable	No n = 716	Yes n = 723	*P*
Age (years)	41 ± 6	56 ± 7	0.0001
BMI (kg/m²)	24.58 ± 5.06	26.13 ± 5.21	0.0001
Diabetes (%)	0.9	3.6	0.001[a]
Hypertension (%)	10.7	33.4	0.0001[a]
CHD (%)	1.1	5.2	0.0001[a]
β-Blocker users (%)	3.9	9.2	0.0001[a]
Cigarette smokers (%)	30.5	29.6	NS[a]
>One drink alcohol/week (%)	64.8	62.1	NS[a]
Triglycerides (mg/dl)	83 ± 50	124 ± 84	0.0001
Total cholesterol (mg/dl)	193 ± 35	229 ± 42	0.0001
VLDL cholesterol (mg/dl)	18 ± 12	27 ± 20	0.0001
LDL cholesterol (mg/dl)	118 ± 33	146 ± 38	0.0001
Non-HDL cholesterol (mg/dl)	135 ± 37	173 ± 45	0.0001
HDL cholesterol (mg/dl)	57 ± 14	57 ± 16	NS
Apolipoprotein B (mg/dl)	85 ± 26	105 ± 32	0.0001
TC/HDL cholesterol	3.60 ± 1.20	4.35 ± 1.53	0.0001
LDL particle score	2.34 ± 0.91	2.68 ± 1.14	0.0001

Values are expressed as mean ± SD.
[a]Chi-square test.

TABLE 9. Pearson correlation coefficients of physical characteristics and plasma lipids with LDL cholesterol and apoB

	Men		Women	
Variable	LDL-C	ApoB	LDL-C	ApoB
Age	0.17[a]	0.17[a]	0.36[a]	0.32[a]
BMI	0.13[a]	0.21[a]	0.16[a]	0.24[a]
Systolic blood pr.	0.10[b]	0.17[a]	0.28[a]	0.31[a]
Diastolic blood pr.	0.12[a]	0.18[a]	0.20[a]	0.21[a]
Glucose	0.01	0.10[b]	0.14[a]	0.25[a]
Triglycerides	0.14[a]	0.49[a]	0.36[a]	0.56[a]
Total cholesterol	0.80[a]	0.65[a]	0.90[a]	0.70[a]
VLDL cholesterol	0.08[c]	0.43[a]	0.27[a]	0.45[a]
LDL cholesterol		0.62[a]		0.74[a]
Non-HDL cholesterol	0.79[a]	0.71[a]	0.88[a]	0.79[a]
HDL cholesterol	−0.11[a]	−0.32[a]	−0.18[a]	−0.32[a]
ApoB	0.62[a]		0.74[a]	
LDL particle score	0.08[c]	0.42[a]	0.16[a]	0.43[a]

[a] $P < 0.0001$.
[b] $P < 0.001$.
[c] $P < 0.05$.

TABLE 10. Stepwise regression analyses of LDL cholesterol and apoB

	Partial r^2	P
Men		
LDL cholesterol $r^2 = 0.059$		
Age	0.036	0.0001
Triglycerides	0.013	0.0001
BMI	0.007	0.002
Cigarette smoking[a]	0.002	0.06
Non-HDL cholesterol $r^2 = 0.345$		
Triglycerides	0.325	0.0001
Age	0.018	0.0001
BMI	0.004	0.003
Blood pressure	0.002	0.05
ApoB $r^2 = 0.255$		
Triglycerides	0.240	0.0001
Age	0.008	0.0002
BMI	0.004	0.01
Cigarette smoking[a]	0.003	0.05
Women		
LDL cholesterol $r^2 = 0.214$		
Triglycerides	0.131	0.0001
Age	0.062	0.0001
Menopausal status[b]	0.010	0.0001
Cigarette smoking[a]	0.006	0.002
Blood pressure	0.005	0.002
Non-HDL cholesterol $r^2 = 0.430$		
Triglycerides	0.368	0.0001
Age	0.050	0.0001
Menopausal status[b]	0.007	0.0001
Blood pressure	0.003	0.02
Cigarette smoking[a]	0.002	0.05
ApoB $r^2 = 0.347$		
Triglycerides	0.308	0.0001
Age	0.021	0.0001
Blood pressure	0.006	0.001
Cigarette smoking[a]	0.006	0.001
Glucose	0.004	0.01
Menopausal status[b]	0.002	0.05

[a] Cigarette smoking: 0 = non smoker; 1 = smoker.
[b] Menopausal status: 0 = premenopause; 1 = postmenopause.

of non-HDL cholesterol and apoB level variability than LDL variability in the Framingham Offspring population. In women, menopausal status was an important determinant of these plasma parameters as well.

DISCUSSION

Cardiovascular disease is the most important cause of morbidity and mortality in industrialized countries (32). Cigarette smoking, hypertension, diabetes mellitus, low HDL cholesterol levels, and elevated LDL cholesterol levels have all been designated as independent risk factors for CHD (7, 33). In clinical practice, total cholesterol is measured in serum, and LDL cholesterol is calculated from total serum cholesterol using the Friedewald formula. In our population, serum total cholesterol and calculated LDL cholesterol levels were very similar to those found in the second National Health and Nutrition Examination Survey, by both age and gender (34). While the Centers for Diseases Control (CDC) have long provided reference material for the standardization and calibration of cholesterol measurement (35), efforts are still under way to establish an international standardization program for apoB (36). Because of the lack of an international standardization program, it is difficult to compare the apoB levels described in the Framingham Offspring population with the apoB values reported by other studies, or to ascribe the observed differences to differences in age and environmental factors or genetic backgrounds of the other populations studied (37–41). However, our assay was calibrated against LDL-apoB standards whose protein concentration had been accurately measured by amino acid analysis.

In our study, aging was associated with higher levels of LDL cholesterol and apoB levels in both men and women. Similar results have been observed in the original Framingham Study cohort (14, 42). Kinetic studies of LDL-apoB in a group of subjects with a broad age range have indicated that the catabolism of LDL decreases with increasing age (43). A reduction of the activity of the LDL receptors in the liver is likely to be responsible for this age-related impairment of LDL catabolism and for the increase in LDL cholesterol levels in men and women. However, after adjustment of LDL cholesterol and apoB levels for age and BMI, these parameters were still significantly higher in postmenopausal women than in premenopausal women, indicating that other factors, independent of age and BMI, strongly influence LDL cholesterol levels in women. The decrease in plasma estrogen levels after menopause may play a significant role in the reduction of the clearance of LDL particles and subsequent increase in LDL cholesterol and apoB levels in postmenopausal women. Estrogen replacement treatment has been shown to markedly decrease both LDL cholesterol (44, 45) and apoB (44) levels in dyslipidemic postmenopausal women. In addition, studies in rats have shown that estrogen treatment is followed by a marked increase in the number of hepatic cell surface LDL receptors and a faster clearance of LDL particles (46). Furthermore, treatment with estrogen has been shown to increase cholesterol excretion in humans (47) and to decrease the conversion of VLDL-apoB to LDL-apoB in rabbits (48).

As already previously reported, we found LDL particle size in men to be significantly smaller than in women (25, 26). We also found that LDL particle size was significantly smaller in postmenopausal women than in premenopausal women. However, this difference was no longer significant after LDL particle score was adjusted for age, BMI, and plasma triglyceride level effects, which have been shown to be very important determinants of LDL particle size (25). These results suggest that the decrease in LDL particle size after menopause is for the most part due to the changes in BMI and triglyceride levels experienced after menopause. Estrogens do not play a major role in the change of LDL particle size associated with menopause, as also suggested by Campos et al. (49). In a selected sample of premenopausal and postmenopausal women from this population we have previously found that postmenopausal women were more likely to have small LDL particles than premenopausal women after adjustment for age, BMI, and plasma triglyceride, LDL cholesterol, and HDL cholesterol levels (50). This discrepancy may be the result of a difference in the expression of LDL particle size in the two studies: while only the major LDL band was taken into account in the previous study (50), both the major and the minor LDL bands were taken into account in the present report.

The NCEP Expert Panel has provided guidelines for the classification and treatment of lipid disorders in adults (7). According to these guidelines, an LDL cholesterol level below 130 mg/dl is considered desirable, an LDL cholesterol level greater than or equal to 160 mg/dl is defined as a high risk factor for CHD, whereas an LDL cholesterol level between 130 and 159 mg/dl is considered a borderline high-risk value. Using the Friedewald formula and serum total cholesterol values, 33% of male and 28% of female participants fall in the high-risk category and therefore, according to these guidelines, would require lipid-lowering treatment. The percentage of subjects in the high-risk category increases substantially with age, and more markedly in women after menopause, when more than 40% of women have LDL cholesterol levels $\geq$ 160 mg/dl. These observations, combined with findings from the Framingham Heart Study suggesting that there is a decreased impact of LDL cholesterol levels on CHD mortality with aging (51), indicate the need for more specific guidelines for the treatment of elevated levels of LDL cholesterol concentrations in the elderly.

In our study, plasma LDL cholesterol levels did not dis-

criminate male subjects with CHD from subjects without CHD. This is in contrast with the results of the Framingham Heart Study that have prompted the NCEP guidelines (2). However, it should be noted that, in the case of the Framingham Heart Study, the predictive effect of LDL cholesterol levels on CHD was determined longitudinally. Similar to our study, other cross-sectional studies have found little or no difference in LDL cholesterol levels between CHD and CHD-free male subjects when LDL concentrations were unadjusted (52–54). A very likely explanation for the findings of these cross-sectional studies, as opposed to those of longitudinal studies, is that subjects with manifest CHD are likely to have changed their dietary habits and life-style and to be taking medications, and thus have modified their plasma lipid levels. It is known that diet modification and use of β-blockers may alter LDL cholesterol concentrations (55, 56). In fact, when LDL cholesterol levels of CHD subjects had been adjusted for diet and β-blocker effects, LDL cholesterol concentrations were significantly higher in CHD than in control subjects (52, 57). However, no dietary information was available on our subjects and after deletion from the analysis of subjects that were on β-blockers, no improvement in CHD prediction on the basis of LDL cholesterol levels was observed. This may be due to the fact that, in the Framingham Offspring population, subjects taking β-blockers were evenly distributed across all LDL cholesterol levels. In contrast to men, LDL cholesterol levels were significantly higher in women with CHD than in CHD-free women (see Tables 6 and 7). This difference is likely due to the confounding effect of age: a much stronger association between age and LDL cholesterol was observed in women than in men. As women with elevated LDL cholesterol tend to be older, they also have a higher chance to have developed CHD.

Our results indicate that elevated apoB levels are associated with CHD in both men and women, in spite of the lack of a similar association between LDL cholesterol and CHD in men. This is in agreement with the findings of previous studies (52–54). Previous cross-sectional studies have suggested that the measurement of plasma apoB levels may provide more information about CHD risk than LDL cholesterol levels alone (9–11). The association between apoB and CHD was very similar to that of non-HDL cholesterol and CHD. The stronger association of CHD with non-HDL cholesterol than with LDL cholesterol alone may underline the role of other non-HDL lipoproteins in the pathogenesis of CHD. Both VLDL and Lp[a] have been implicated in the development of CHD, even though different mechanisms of atherogenesis have been suggested for these lipoproteins (58–60). While most studies have indicated that VLDL particles are only indirectly associated with CHD, some authors suggest that VLDL may have a direct atherogenic effect independent from their relationship with HDL cholesterol levels and metabolism (38). Even though our study suggests that apoB levels may be better indicators of CHD risk than LDL cholesterol levels, only a longitudinal analysis of this population will provide a definite answer. In fact, results from the Physician's Health Study (12) indicate that apoB has little predictive value for CHD risk in males after taking into account standard non-lipid risk factors and the total cholesterol/HDL cholesterol ratio, suggesting that the balance between non-HDL cholesterol and HDL cholesterol may play a more important role in the development of CHD than apoB alone.

The variable most associated with CHD was the total cholesterol to HDL cholesterol ratio. This is in agreement with the results of the Physicians' Health Study (12) which showed that the ratio of total to HDL cholesterol was the parameter most predictive of myocardial infarction. According to our data, TC/HDL ratio values above 5 may be considered as a high risk value for CHD, in both men and women.

Even though plasma levels of both LDL cholesterol and apoB are highly heritable (61, 62), environmental factors affect plasma concentrations of these parameters. In previous studies it has been shown that diet composition, and particularly dietary cholesterol and saturated fatty acids, have a significant effect on LDL cholesterol (55). We found that, among other environmental factors, the most important determinants of LDL cholesterol, non-HDL cholesterol, and apoB levels are age, BMI, and plasma triglyceride levels. In women, menopause is an additional determinant of these parameters. Blood pressure was significantly associated with these parameters in women and with non-HDL cholesterol in men after adjustment for the effects of age, BMI, and triglyceride levels. This relationship, though not very strong, may reflect the presence in our population of subjects with familial dyslipidemic hypertension, a heritable disorder characterized by the association of alteration in plasma lipid levels with essential hypertension (63, 64). The association of plasma LDL cholesterol, non-HDL cholesterol, and apoB levels with BMI is very important in the light that body weight is a factor that can be modified. The strong association between BMI and LDL cholesterol levels was also seen when subjects were divided on the basis of their plasma LDL cholesterol levels (see Table 6). Our results support the findings of Denke, Sempos, and Grundy (65) who found a consistent increase in plasma LDL cholesterol and non-HDL cholesterol levels with increasing BMI in different age groups of men participating to the second National Health and Nutrition Examination Survey.

Clearly, the measurement of apoB concentrations in the plasma is of great interest. Even though our results suggest that apoB and non-HDL cholesterol levels provide more information about CHD risk than LDL cholesterol levels, these findings need to be confirmed in

large prospective studies. Our data from the Framingham Offspring Study will provide such information in the future.

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