LDL Particle Size Distribution

Results From the Framingham Offspring Study

Hannia Campos, Erling Blijlevens, Judith R. McNamara, José M. Ordovas, Barbara M. Posner, Peter W.F. Wilson, William P. Castelli, and Ernst J. Schaefer

Using 2–16% gradient gel electrophoresis, we examined low density lipoprotein (LDL) particle size in relation to plasma lipoproteins in 1,168 women and 1,172 men from the Framingham Offspring Study. In addition, we studied the effect of dietary intake on LDL size in a subset of the population. Seven LDL size peaks were identified, with the largest, LDL 1, being found in the density range 1.019–1.033 g/ml; LDL 2 and LDL 3 in d=1.033–1.038 g/ml; LDL 4 and LDL 5 in d=1.038–1.050 g/ml; and the smallest, LDL 6 and 7, in d=1.050–1.063 g/ml. Seventy-seven percent of the population had one major and at least one minor LDL peak. Secondary LDL peaks accounted for 23% of the total LDL relative area, based on laser scanning densitometry. LDL size distribution was skewed toward larger LDL particles in women (prevalence of LDL 1, 30% and of LDL 2, 31%), whereas men exhibited a more symmetric distribution (prevalence of LDL 3, 42%). The prevalence of small (<255 Å), dense (d>1.038 g/ml) LDL particles 4–7 was 33% in men, 5% in premenopausal women, and 14% in postmenopausal women. In agreement with previous reports, small, dense LDL particles were significantly (p<0.0001) associated with increased triglyceride and apolipoprotein (apo) B levels and decreased HDL cholesterol and apo A-I levels. In addition, we found a significant (p<0.0001) association between LDL cholesterol and LDL size. The highest LDL cholesterol levels were found among women with LDL 4 (148 mg/dl) and men with LDL 3–5 (138 mg/dl). In addition, the presence of LDL 3 or 4 as secondary peaks was significantly associated with higher LDL cholesterol levels, while smaller secondary LDL peaks were associated with higher triglyceride levels. We also found that compared with subjects with optimal LDL cholesterol levels (<130 mg/dl), individuals with high-risk LDL cholesterol levels (≥160 mg/dl) had 1) a higher prevalence of LDL 3 and 4 (women only) and a lower prevalence of LDL 1 and 2 (women only) and 2) 11% higher LDL cholesterol to apo B ratios, even when matched for LDL particle size. Furthermore, low saturated fat and cholesterol intakes were significantly associated (p<0.01) with smaller LDL particles. Therefore, the identification of small, dense LDL particles per se may not be a good indicator of coronary artery disease risk in population studies. Gender differences and environmental factors that affect triglyceride levels and LDL physical and chemical properties should be taken into consideration. In addition, LDL 3–5 particles in men and 4 in women are associated with the highest LDL cholesterol levels. (*Arteriosclerosis and Thrombosis* 1992;12:1410–1419)

KEY WORDS • LDL particle size • cholesterol • plasma lipoproteins • triglycerides • gradient gel electrophoresis • apolipoproteins • dietary fat intake

Low density lipoproteins (LDL) are the major cholesterol-carrying lipoproteins in plasma.[1] LDL isolated in the density region of 1.019–1.063 g/ml contain approximately (weight percent) 50% cholesterol (free and esterified), 25% protein, 20% phospholipid, and 5% triglyceride.[1] Apolipoprotein (apo) B, a 550-kd polypeptide, comprises over 95% of LDL protein mass.[2] Elevated plasma levels of LDL cholesterol and apo B have been associated with premature coronary artery disease (CAD).[3–5]

LDL particles are heterogeneous in size and density.[6] On gradient gel electrophoresis, seven LDL subgroups can be identified[6–8] and have been shown to correlate with specific LDL subclass density ranges.[6,8,9] These LDL subclasses have been classified into two LDL phenotypes (patterns A and B).[10] Several studies indicate that these two LDL phenotypes are inherited as a single-gene trait with a dominant mode of inheritance.[10–12] Other studies suggest that LDL subclasses are strongly influenced by environmental factors.[13–18] The predominance of small (diameter <255 Å), dense (d>1.038 g/ml) LDL particles has been associated with the presence of myocardial infarction (MI)[19] and CAD,[20–22] but this association is not independent of triglyceride levels[19,20] or established cardiovascular risk factors, particularly LDL and high density lipoprotein (HDL) cholesterol levels.[21,22]

From the Lipid Metabolism Laboratory (H.C., E.B., J.R.M., J.M.O., E.J.S.), US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, Mass.; the Framingham Heart Study (B.M.P., P.W.F.W., W.P.C.), National Heart, Lung, and Blood Institute, Framingham, Mass.; and the Schools of Medicine and Public Health (B.M.P.), Boston University, Boston, Mass.

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Address for correspondence: Dr. Ernst J. Schaefer, Lipid Metabolism Laboratory, USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111.

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It is well known that small, dense LDL particles are associated with increased triglyceride and apo B levels and decreased HDL cholesterol and apo A-I levels.[8,9,12] These associations have been observed in several normal populations,[8,9,16] in men with CAD,[19,20,22] and in familial combined hyperlipidemia.[11] However, the association between LDL subclasses and LDL cholesterol levels is unclear. While some studies indicate that the predominance of small, dense LDL particles is associated with significantly higher LDL cholesterol levels,[8,12] other studies have reported either no association[9,11] or a trend toward lower LDL cholesterol levels in subjects with small, dense LDL.[19,22] Furthermore, cross-cultural observations indicate that while small, dense LDL particles are more prevalent among populations with lower LDL cholesterol levels and lower dietary fat intake than those in the United States,[16] these particles are rarely found among study subjects from The Netherlands,[9] a country where dietary fat consumption is high.[23]

In the present study we examined the distribution of LDL subclasses, as well as the presence of secondary LDL peaks, in relation to plasma lipoproteins, particularly LDL cholesterol levels, in 1,172 men and 1,168 women from the Framingham Offspring Study. In addition, we studied the association between dietary intake and LDL particle size in a subset of this population.

Methods

Study Subjects

The study subjects were 1,172 men and 1,168 women in cycle 3 of the Framingham Offspring Study who had complete data on LDL particle size and biochemical parameters. Subjects were free of MI or CAD and were not taking β-blockers, oral contraceptives and/or estrogen, or any other medications known to affect lipids. All subjects had blood drawn after a 12-hour fast as part of an approved protocol as previously described.[3]

LDL Subclass Determination

LDL subclasses were separated by subjecting whole plasma to 2–16% gradient gel electrophoresis (PAA 2–16%, Pharmacia, Piscataway, N.J.) and were visualized by using Sudan black to stain the LDL particles, as previously described.[8] Scanning was performed on an LKB Ultrascan XL laser densitometer (LKB Instruments Inc., Paramus, N.J.) interfaced with an AT&T computer (LKB) and a Canon PJ-108A printer using the LKB GSXL software for peak integration. Each subject was assigned an LDL type, with the largest, LDL 1, being found in the density range 1.019–1.033 g/ml; LDL 2 and LDL 3 in the range 1.033–1.038 g/ml; LDL 4 and LDL 5 in the range 1.038–1.050 g/ml; and the smallest, LDL 6 and 7, in the range 1.050–1.063 g/ml. Since we previously found that 88% of subjects have one major peak and one or two minor peaks, we estimated the percent relative area of each LDL peak after scanning. The identification of each LDL peak was based on their relative distance from standard peaks of a known plasma sample that was run in duplicate in each gel.[8] Both migration distance and the percent total area for each peak have been shown to remain constant through a large LDL concentration range and from gel to gel.[8] Therefore, misclassification due to staining and/or sample concentration is minimal. In this study we found that 35% of the population had secondary LDL peaks of at least 30% of the total area and that 77% had one major and at least one minor secondary LDL peak. To take into consideration the presence of secondary peaks, an LDL score for each subject was calculated as the sum of the relative areas under all LDL peaks present. The boundaries for each LDL peak within the total LDL particle distribution were selected at the points of inflection. The baseline was set at background on both sides of the LDL band range. Figure 1 (top panel) shows a representative scan for two women with predominant LDL 1 and LDL 2 and LDL particle scores of 1.44 and 1.94 and for two men (lower panel) with a predominant LDL 3 and LDL scores of 2.59 and 3.16. For example, for the first male subject with a predominant LDL 3 (59% of area) and a secondary LDL 2 (41% of area), an LDL score of 2.59 was calculated [(3×0.59)+(2×0.41)]. For the second male subject with the same predominant LDL 3 (84% of area) but a smaller secondary LDL 4 (16%), an LDL score of 3.16 was calculated [(3×0.84)+(4×0.16)]. A smaller LDL particle score corresponds to a larger LDL particle diameter.

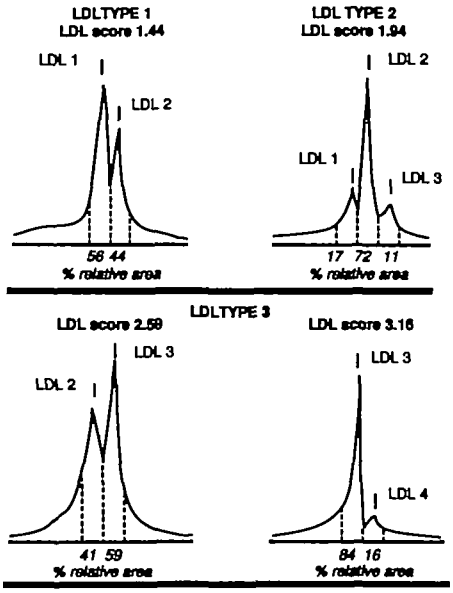

FIGURE 1. Top panel: *Representative scans for women with predominant low density lipoprotein (LDL) 1 and LDL 2 and LDL scores of 1.44 [(1×0.56)+(2×0.44)] and 1.94 [(1×0.17)+(2×0.72)+(3×0.11)].* Lower panel *shows representative scans for two men with a predominant LDL 3 but with different secondary peaks. The lower LDL score of 2.59 [(2×0.41)+(3×0.59)] indicates the presence of larger LDL particles compared with the higher LDL score of 3.16 [(3×0.84)+4×0.16)].*

Lipoprotein and Apolipoprotein Analyses

Blood was drawn from subjects after a 12–14-hour fast in 0.15% EDTA (final concentration), and plasma samples were centrifuged at 2,500 rpm for 20 minutes at 4°C to isolate plasma. Plasma was subjected to ultracentrifugation at $d=1.006$ g/ml for 18 hours at 39,000 rpm, and the 1.006 g/ml supranatant and infranatant fractions were isolated. The HDL supernate was obtained

Table 1. LDL Subclass Distribution in Women and Men From the Framingham Offspring Study and Corresponding LDL Subgroup Classification as Previously Reported†

	This study										Other studies				
	LDL peak area distribution (%)							*n* (%)							
LDL type*	1	2	3	4	5	6	7	F	M	Mean LDL score	LDL subclasses (g/ml)†	LDL particle diameter (Å)‡	LDL subclass pattern§	LDL subfractions (g/ml)‖	LDL score groups#
1	64.6	24.5	9.9	0.6	0.2	0.1	0.1	348 (30)	84 (7)	1.48±0.32	LDL I 1.025–1.032	260–275	A	LDL-1 1.020–1.028	Large
2	9.2	74.9	13.4	1.8	0.2	0.2	0.2	364 (31)	200 (17)	2.11±0.35	LDL IIA 1.030–1.038	255–270	A	LDL-2 1.027–1.034	Large
3	1.6	6.6	76.2	14.3	0.7	0.2	0.2	345 (29)	495 (42)	3.07±0.32	LDL IIB 1.035–1.040			LDL-3 1.033–1.039	Intermediate
4	0.2	0.9	14.7	73.4	10.0	0.2	0.2	47 (4)	150 (13)	3.92±0.34	LDL IIIA 1.038–1.048	247–252	B	LDL-4 1.039–1.049	Small
5	0.3	0.2	5.1	10.6	80.7	2.0	1.1	53 (5)	202 (17)	4.82±0.33	LDL IIIB 1.038–1.048	242–246			Small
6	0.0	0.2	0.3	3.8	2.3	85.7	7.7	9 (1)	39 (3)	6.00±0.30	LDL IVA 1.048–1.065	233–242		LDL-5 1.049–1.061	Very small
7	0.0	0.0	0.0	0.0	12.5	6.0	81.5	2 (0.2)	2 (0.2)	6.68±1.53	LDL IVB 1.048–1.065	218–232	B		
								1,168 (100)	1,172 (100)						

LDL, low density lipoprotein.
*As defined by McNamara et al.[8]
†As defined by Nichols et al.[7]
‡As defined by Krauss.[27]
§As defined by Austin et al.[10]
‖As defined by Swinkels et al.[9]
#As defined by Campos et al.[22]

after precipitation of very low density lipoprotein and LDL with dextran–magnesium sulfate by using the method of Warnick et al.[24] Plasma total cholesterol, triglyceride, 1.006 g/ml infranatant cholesterol, and HDL cholesterol levels were determined enzymatically with an Abbott Diagnostics ABA-200 bichromatic analyzer and Abbott A-GENT reagents.[25] Plasma apo A-I and apo B levels were determined with a noncompetitive enzyme-linked immunosorbent assay as previously described.[26]

Dietary Assessment and Statistical Analysis

To examine the association between dietary intake and LDL subclasses within a population, dietary information was obtained from a subsample of 85 women and 76 men. Dietary intake was assessed by a self-administered food-frequency questionnaire as previously described.[16] The mean dietary intake for this population has been previously reported.[16] Statistical analyses were performed with the Statistical Analysis Systems software (SAS, Cary, N.C.). The procedures included *t* test analysis for mean comparisons of lipoprotein and apolipoprotein plasma parameters in women and men. The general linear model procedure and the least-square-means option were used for the LDL subclass analysis of variance, covariance, and age adjustments. The LDL particle score distribution plots and Pearson correlation coefficients were carried out by using the Chart, Corr, Freq, and Univariate procedures in the SAS system.

Results

LDL Subclass Distribution in Women and Men

The population distribution for the seven LDL types, mean percent LDL peak area distribution, and mean LDL particle score (LDL subclass classification, in which secondary LDL peaks are taken into consideration) for all women and men in this study, as well as the corresponding LDL subclass classifications from previous reports, are shown in Table 1. LDL 1 and 2 were found most frequently among women. LDL 3 was the most frequently found LDL size among men. In men, 33% had small LDL 4–7, and in women this percentage was 10%. It should be pointed out that 45% of the women in this population were postmenopausal, and that menopausal status was significantly associated with LDL particle size distribution. The prevalence of small LDL 4–7 was 5.3% in premenopausal compared with 14.3% in postmenopausal women ($\chi^2<0.0001$). Since this difference was no longer significant after adjusting for age, we included all women as one group, and all data were age adjusted.

Percent LDL peak area for each predominant LDL size ranged between 65% and 86% of the total peak area, while adjacent secondary peaks accounted for a range between 2% and 25% of the total peak area. Combinations among LDL subclasses that were more than two LDL types apart (e.g., LDL 1 and LDL 4) were very rarely found (distant peaks accounted for <0.6% of area). The LDL score distribution for each LDL type is shown in Figure 2. The LDL score populations are normally distributed, with LDL 5 skewed toward larger LDL particles. Since the calculated LDL scores are based on the percent peak area distribution, LDL scores that represent more than one LDL type apart from the predominant LDL subclass (e.g., a predominant LDL 1 with and an LDL score ≥3.0) were uncommon (0.4% of the subjects).

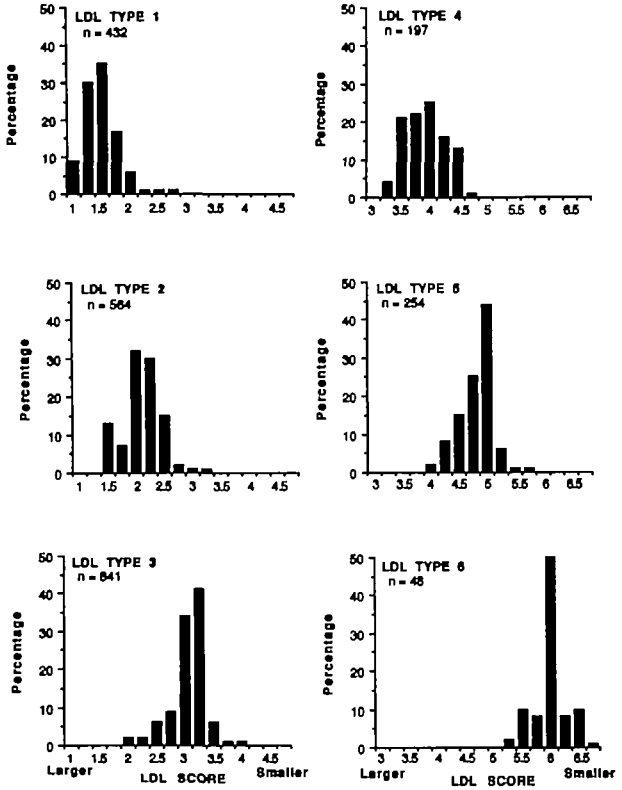

FIGURE 2. *Bar graphs show the percent distribution of low density lipoprotein (LDL) scores for six predominant LDL peaks found among 1,168 women and 1,172 men from the Framingham Offspring Study.*

To test whether the presence of secondary peaks was associated with lipoprotein parameters, we examined associations between LDL score and plasma triglyceride, HDL cholesterol, and LDL cholesterol levels by LDL type group, adjusted for age and gender, in women and men. As shown in Table 2, secondary peaks in subjects with LDL 1 and LDL 2 were not associated with triglyceride or LDL cholesterol. In subjects with LDL 1, the presence of a smaller, secondary LDL peak was significantly associated with higher HDL cholesterol levels. This LDL subclass profile was commonly found among women (see top panel of Figure 1 and Figure 3A). The presence of secondary LDL peaks was most informative for subjects with LDL type 3 (mostly found in men; see Figure 3B). In this group lower, large, secondary peaks (e.g., LDL 1 or 2) and the presence of smaller secondary peaks (e.g., LDL 4; see lower panel of Figure 1) were associated with increased triglyceride and LDL cholesterol levels and decreased HDL cholesterol levels. A reduction in large, secondary LDL peak area in subjects with LDL types 4, 5, and 6 was also associated with increased triglyceride levels but not with HDL cholesterol levels. A reduction in large, secondary LDL peaks in subjects with predominant LDL 4, 5, and 6 was associated with lower LDL cholesterol levels. It should be noted that in this last group of subjects, "larger" LDL peaks corresponded mostly to large, secondary peaks of LDL 3 and 4 and not to LDL 1 and 2 (see Table 1 for percent peak area distribution). Therefore, the presence of LDL 3 and 4 as secondary peaks is associated with higher LDL cholesterol levels.

TABLE 2. Correlation Coefficients Between LDL Particle Score and Lipids by LDL Type

LDL type	*n*	TG	HDL chol	LDL chol
LDL 1	432	−0.02	0.13*	−0.10
LDL 2	564	−0.03	−0.01	0.07
LDL 3	840	0.32‡	−0.38‡	0.23‡
LDL 4	197	0.16	−0.02	−0.08
LDL 5	259	0.46‡	−0.06	−0.14*
LDL 6	48	0.29*	−0.26	−0.35†

LDL, low density lipoprotein; TG, triglyceride; chol, cholesterol. Analysis was carried out on data for all men and women (n=2,340), with values adjusted for age and gender.
*p<0.05, †p<0.01, ‡p<0.0001.

Figures 3A and 3B show the LDL score distribution in women and men. The LDL particle score distribution in women is skewed toward larger particles (skewness=1.0), as expected by a higher prevalence of women with predominant LDL 1 and LDL 2, while in men the LDL score distribution is more symmetric (skewness=0.3) because of a predominance of men with LDL 3. The 10th, 25th, 75th, and 90th percentiles for LDL particle scores are 1.35, 1.60, 3.00, and 3.60 in women and 2.00, 2.60, 4.00, and 5.00 in men, respectively. These percentiles in men correspond with the LDL score cutpoints that were previously used to identify large, intermediate, and small LDL groups[22] (also see Table 1 for comparison with other LDL subclass classification systems). Figure 3C shows the LDL distribution in all subjects as well as the mean LDL scores for women and men in this study. For comparison with previous reports of LDL particle size in population studies, we have provided the mean LDL scores for men and women from a healthy Costa Rican population who habitually consume a low-fat diet. Both women and men from this population have been reported to have significantly smaller LDL particles than do subjects from the United States.[16] In addition, we have included the mean LDL score previously reported for patients with CAD.[22] As shown in this figure, the mean LDL score in these patients is identical to that previously reported for the Costa Rican group.

LDL Subclasses and Plasma Triglyceride, HDL Cholesterol, and Apolipoproteins

Mean plasma lipoprotein and apolipoprotein concentrations, as well as mean LDL particle score and type for women and men, are shown in Table 3. Men had significantly (p<0.0001) higher triglyceride, LDL cholesterol, and apo B levels and significantly lower HDL cholesterol and apo A-I levels compared with women. Mean LDL particle score and mean LDL particle type were 3.33 and 3.26 for men and 2.42 and 2.26 for women, respectively (a higher value was obtained when LDL scores were used instead of LDL type, indicating a predominance of smaller rather than larger secondary peaks when these are taken into consideration). No significant differences in age and total cholesterol were found between women and men.

The plasma parameters for the seven LDL types in women are shown in Table 4. By analysis of variance the associations of all plasma parameters with LDL type were significant (p<0.0001) after adjusting for age.

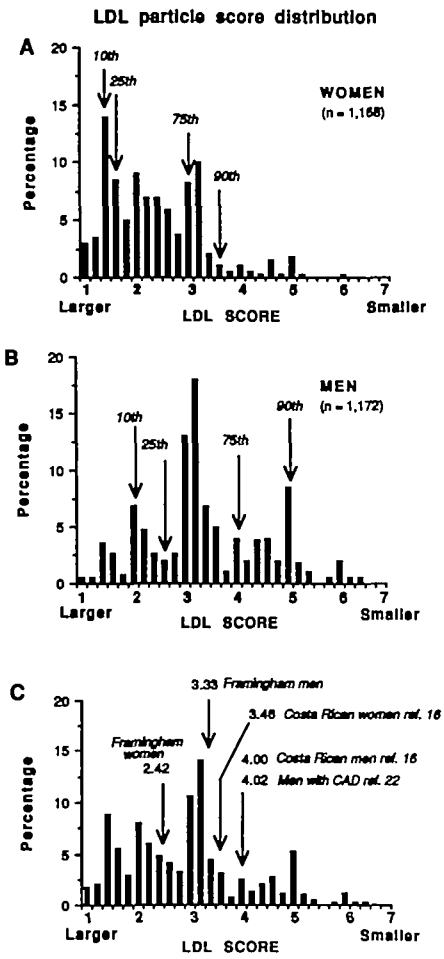

FIGURE 3. *Bar graphs show the percent low density lipoprotein (LDL) particle size distribution in* (panel A) *women and* (panel B) *men from the Framingham Offspring Study when secondary LDL peaks are taken into consideration (LDL scores). Arrows indicate the 10th, 25th, 75th, and 90th percentiles. In women these percentiles correspond to LDL scores of 1.35, 1.60, 3.00, and 3.60 and in men of 2.00, 2.60, 4.00, and 5.00, respectively.* Panel C: *Bar graph shows the LDL score distribution in all Framingham subjects (n=2,340). Arrows indicate the mean LDL score for men and women in this study, as well as previously reported LDL score means for a healthy Costa Rican population who habitually consume a low fat-diet and for patients with coronary artery disease.*

Triglyceride levels were significantly lower in the large-LDL subclasses. Triglyceride levels increased with decreasing LDL size, with the smallest, LDL 7, having a mean triglyceride level of 632 mg/dl. However, only two subjects were found in this last category. The difference in triglyceride levels between all LDL particle types was statistically significant ($p<0.001$). Apo B levels were also lower in the large LDL particle types and increased with decreasing size. However, no significant differences were found within LDL types 4, 5, 6, and 7. These three groups had the highest apo B levels (mean, 122 mg/dl). In contrast, HDL cholesterol and apo A-I levels decreased with decreasing size. No significant differences in HDL cholesterol were found between LDL score groups 4, 5, 6, and 7. These groups had the lowest HDL cholesterol levels (mean, 39 mg/dl). Similar results were obtained for apo A-I levels. The plasma parameters for the seven LDL types in men shown in Table 5 are similar to the values observed in women, except for those shown in previous tables, where there is a higher prevalence of men with smaller LDL particles. By multivariate analysis, triglyceride (42%) and HDL cholesterol (8%) levels accounted for 50% of the variance in LDL particle size in women and for 57% of the variance in LDL size in men.

TABLE 3. Mean Lipoprotein and Apolipoprotein Levels in Women and Men From the Framingham Offspring Study

Parameter	Women (n=1,168)	Men (n=1,172)	p
Age (years)	48.0±9.6	47.7±10.0	0.4
Total cholesterol (mg/dl)	207±41	209±38	0.1
Triglyceride (mg/dl)	94±71	134±102	0.0001
LDL cholesterol (mg/dl)	128±37	134±34	0.0001
HDL cholesterol (mg/dl)	57±14	45±12	0.0001
Apo B (mg/dl)	92±30	107±33	0.0001
Apo A-I (mg/dl)	158±37	136±33	0.0001
LDL particle score*	2.42±1.00	3.33±1.14	0.0001
LDL type†	2.26±1.13	3.26±1.24	0.0001

LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein.

*LDL particle score for each subject was calculated as the sum of the relative areas under all the LDL bands present (see text for details).

†LDL type represents the predominant LDL peak observed in each subject. A lower score or type represents a larger LDL particle.

LDL Subclasses and LDL Cholesterol Levels

The associations of LDL cholesterol and LDL type were different from those observed for other lipoprotein parameters (Tables 4 and 5). LDL cholesterol levels did not increase or decrease consistently with LDL particle size, as observed for triglyceride, apo B, HDL cholesterol, and apo A-I. The highest LDL cholesterol levels were found in women with LDL type 4 (mean, 148 mg/dl) and in men with LDL types 3, 4, and 5 (mean, 138 mg/dl). The lowest LDL cholesterol levels were found in women with LDL 1, 2, and 7 (mean, 126 mg/dl) and in men with LDL 1, 6, and 7 (mean, 118 mg/dl). LDL cholesterol in an intermediate range was found in women with LDL 3, 5, and 6 (mean, 132 mg/dl) and in men with LDL 2 (mean, 130 mg/dl). In sum, the associations between LDL cholesterol and LDL subclass distribution are similar in men and women, with the higher LDL cholesterol levels being observed in subjects with intermediate and small LDL-sized particles. The association between LDL cholesterol and LDL subclasses is parabolic, and it is not apparent when Pearson or Spearman correlations or linear regression analysis is used. In addition, when LDL subclasses are divided in two groups, different associations between LDL cholesterol and LDL size are obtained, depending on the sample selection procedures and the prevalence of subjects at the extreme (large or small) LDL subclasses.

To further explore the association between LDL cholesterol and LDL type, we compared LDL type

TABLE 4. Plasma Lipoprotein Levels by LDL Type in Women From the Framingham Offspring Study

Plasma parameter	LDL 1 (n=348)	LDL 2 (n=364)	LDL 3 (n=345)	LDL 4 (n=47)	LDL 5 (n=53)	LDL 6 (n=9)	LDL 7 (n=2)	Analysis of variance p
Total triglyceride	66±3	77±3	103±3	161±8	212±8	334±18	632±29	<0.0001
LDL cholesterol	124±2*	128±2*	132±2†	148±5	133±5†	130±12†	75±19	<0.0001
HDL cholesterol	67±1	58±1	52±1	44±2*	41±2*	33±4*	47±7.0*	<0.0001
Apo B	84±1	88±1	96±1	116±4*	124±4*	127±9*	141±15*	<0.0001
Apo A-I	171±2	159±2	151±2	142±5*	138±5*	118±13*	155±20*	<0.0001

LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein. All parameters were adjusted for women aged 48 years and are given in milligrams per deciliter as mean±SEM. All groups are significantly different from each other (p<0.01) except as indicated (* or †), where groups with the same symbol are not significantly different from each other. By multivariate analysis triglyceride (R^2=0.42) and HDL cholesterol (R^2=0.08) accounted for 0.50 of the variability in LDL size. Apo B, LDL cholesterol, and apo A-I were also independently associated with LDL size (p<0.01) and accounted for 0.02 of the variance in LDL size.

distribution in women and men with high-risk (≥160 mg/dl) and optimal (<130 mg/dl) LDL cholesterol levels, as based on the National Cholesterol Education Program cutpoints.[28] As shown in Figure 4, women in the high LDL cholesterol group displayed a significantly higher prevalence of LDL types 3 and 4 (33% and 11%, respectively) and a lower prevalence of LDL types 1 and 2 (21% and 26%, respectively) relative to those in the low LDL cholesterol group (26% and 2% for LDL 3 and 4 and 35% and 33% for LDL 1 and 2, respectively). When compared with men in the low LDL cholesterol group, men in the high LDL cholesterol group had a significantly higher prevalence of LDL type 3 (48% versus 37%, respectively) and a lower prevalence of LDL type 1 (3% versus 11%, respectively).

Table 6 shows the LDL cholesterol to apo B ratio in women and men in each LDL type group and the high versus low LDL cholesterol groups. As shown in previous studies, the LDL cholesterol to apo B ratio decreased with increasing density in both LDL cholesterol groups. It is important to point out that our apo B assay measures apo B within LDL and triglyceride-rich lipoproteins. However, in all subjects most of the apo B in plasma is found within LDL. The highest LDL cholesterol to apo B ratio was found among subjects with high LDL cholesterol levels and large LDL 1 and LDL 2. When subjects with low and high LDL cholesterol levels were matched for LDL type, those in the high LDL cholesterol group had, on average, an 11% higher LDL cholesterol to apo B ratio than those in the low LDL cholesterol group in all the LDL type groups. Therefore, subjects with high LDL cholesterol levels appear to have cholesterol-enriched LDL particles compared with those with low LDL cholesterol levels, despite their having the same predominant LDL subclass. An increased prevalence of men with LDL 3 and women with LDL 3 and 4 was found in association with LDL cholesterol levels ≥160 mg/dl.

LDL Subclasses and Dietary Intake

Table 7 shows the association between dietary intake and LDL particle score in 161 men and women after adjusting for caloric intake, age, and gender. Small, dense LDL particles were significantly associated with decreased cholesterol intake (r=−0.27, p<0.001). Small LDL particles were also associated with decreased total, saturated, and monounsaturated dietary fatty acids (r=−0.18, p<0.05–0.01), as well as with decreased animal fat consumption (r=−0.21, p<0.01). No association between LDL particle score and protein, carbohydrate, or polyunsaturated fatty acid intake was found. Figure 5 shows the mean cholesterol, animal fat, and saturated fatty acid intake for the lower 25th, the 25th–75th, and the upper 75th percentile groups, as well as the corresponding mean LDL score. Subjects in the lower 25th percentile for dietary cholesterol (91 mg/1,000 kcal), animal fat (13% of calories), and saturated fat (9% of calories) had the smallest LDL particles (mean LDL score±SEM, 3.29±0.08) in all the diet groups. Subjects in the 25th–75th percentile for saturated fat intake (12.5% of calories) had significantly larger LDL particles (mean LDL score±SEM, 2.91±0.1) when compared with those in the lower 25th percentile group. The largest LDL particles (LDL score mean±SEM, 2.70±0.2) were found among subjects in the upper 75th percentile for cholesterol intake (200 mg/1,000 kcal). Significantly (p<0.05) larger LDL particles were also found for

TABLE 5. Plasma Lipoprotein Levels by LDL Type in Men From the Framingham Offspring Study

Plasma parameter	LDL 1 (n=84)	LDL 2 (n=200)	LDL 3 (n=495)	LDL 4 (n=150)	LDL 5 (n=202)	LDL 6 (n=39)	LDL 7 (n=2)	Analysis of variance p
Total triglyceride	54±9	78±5	104±3	151±6	239±5	334±12	582±52	<0.0001
LDL cholesterol	115±4*	130±2	139±2†	140±3†	136±2†	116±5*	124±23*	<0.0001
HDL cholesterol	62±1	53±1	45±4	42±1	37±1*	33±2*	37±7*	<0.0001
Apo B	81±3	94±2	105±1	116±2	127±2*	133±5*	100±21*	<0.0001
Apo A-I	165±4	150±2	135±1*	135±3*	122±2†	118±5†	126±21†	<0.0001

LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein. All parameters were adjusted for men aged 48 years and are given in milligrams per deciliter as mean±SEM. All groups are significantly different from each other (p<0.01) except as indicated (* or †), where groups with the same symbol are not significantly different from each other. By multivariate analysis triglyceride (R^2=0.46) and HDL cholesterol (R^2=0.11) accounted for 0.57 of the variability in LDL size. Apo B, LDL cholesterol, and apo A-I were also independently associated with LDL size (p<0.01) and accounted for 0.02 of the variance in LDL size.

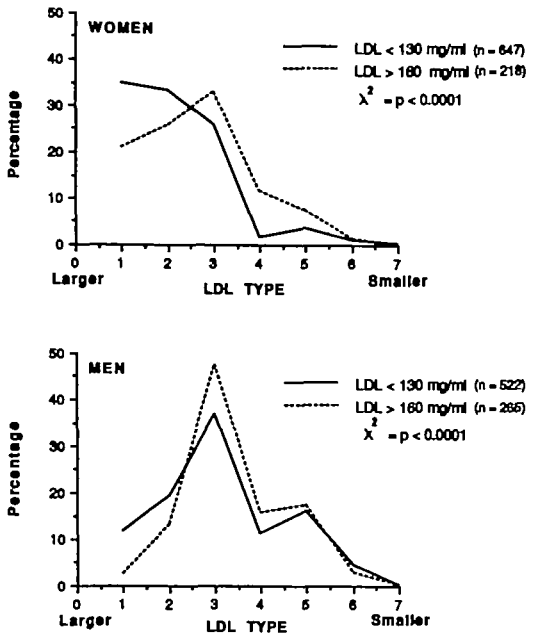

FIGURE 4. *Graphs show percentage of subjects with high-risk (≥160 mg/dl) and optimal (<130 mg/dl) low density lipoprotein (LDL) cholesterol levels for each predominant LDL peak.*

subjects in the upper 75th percentile of animal fat intake (29% of calories).

Discussion

In this study we examined LDL particle size distribution in 1,168 women and 1,172 men from the Framingham Offspring Study to establish normal LDL size ranges and how these relate to diet and plasma lipoproteins in a representative, randomly selected population in the United States. In agreement with previous reports,[8,29] our data indicate that women had a higher prevalence of LDL 1 and LDL 2 compared with men, who usually showed a predominance of LDL 3. The prevalence of small, dense LDL was 33% in men. This finding is consistent with other studies on LDL subclasses among men of comparable age in the United States, where the prevalence of pattern B ranges between 31% and 44%.[10,19] It has been reported that pattern B is inherited as a single-gene trait with a dominant mode of inheritance,[10] which is fully expressed in postmenopausal women with a frequency of 49% compared with 13% in premenopausal women.[10] Our study does not support this finding in women, since there were only 5% of premenopausal and 13% of postmenopausal women with a predominance of small, dense LDL particles in the Framingham population.

LDL subclasses have usually been analyzed in population studies by identification of the predominant LDL peak via gradient gel electrophoresis.[8,12,19] In our study we examined the predominance of secondary LDL peaks and their effect on plasma lipids. Our data indicate that, overall, 77% of the total LDL peak area is accounted for by the predominant LDL peak, 21% of the area is found in adjacent LDL secondary peaks, and less than 2% of the area is found in distant secondary peaks. Furthermore, the presence of secondary LDL peaks provided additional information with regard to the associations between LDL particle size and LDL cholesterol and triglyceride levels. Our data indicate that the presence of LDL 3 and LDL 4 as secondary peaks is associated with higher LDL cholesterol levels, and the presence of smaller LDL peaks is associated with higher triglyceride levels. Additionally, in our study the LDL particle size distribution, as calculated when secondary LDL peaks were taken into consideration, was skewed toward larger LDL particles in women and was more symmetrically distributed in men. Seventy-five percent of women had an LDL score ≤3.0, and 75% of men had a value ≤4.0. We did not find a bimodal distribution of LDL particles, as previously reported when the particle diameter of the predominant LDL peak was used.[30] These differences could be due to the smaller sample size and the analysis of men and women as one group in the previous study.[30]

It has been established that LDL particle size is associated with increased triglyceride, VLDL mass, intermediate density lipoprotein mass, and apo B levels and with decreased HDL cholesterol, HDL_2 cholesterol, and apo A-I concentrations.[8,9,12,27] Our present data are in agreement with these previous observations. HDL cholesterol and apo A-I levels decreased and triglyceride and apo B levels increased consistently with decreased LDL particle size. The association between LDL cholesterol and LDL particle size is more complex. While some studies indicate that higher LDL cholesterol levels are found among subjects with small, dense

TABLE 6. LDL Cholesterol to Apo B Ratio by LDL Type in Women and Men With Low (<130 mg/dl) and High (≥160 mg/dl) LDL Cholesterol Levels

	Women LDL cholesterol/apo B				Men LDL cholesterol/apo B			
	LDL cholesterol <130 mg/dl		LDL cholesterol ≥160 mg/dl		LDL cholesterol <130 mg/dl		LDL cholesterol ≥160 mg/dl	
LDL type	*n*	Ratio	*n*	Ratio	*n*	Ratio	*n*	Ratio
LDL 1	225	1.46±0.02	46	1.63±0.05‡	60	1.39±0.03	7	1.68±0.09†
LDL 2	214	1.45±0.02	56	1.60±0.05†	101	1.37±0.03	35	1.50 ±0.05*
LDL 3	167	1.35±0.02	72	1.48±0.04†	193	1.30±0.02	126	1.43 ±0.02†
LDL 4	10	1.27±0.10	25	1.36±0.07	59	1.15±0.03	42	1.34 ±0.04‡
LDL 5	22	1.08±0.05	16	1.20±0.06	84	1.05±0.03	46	1.17 ±0.04*
LDL 6	6	0.77±0.44	3	2.11±0.60	24	0.78±0.06	8	1.11 ±0.12*

LDL, low density lipoprotein; apo, apolipoprotein.
Significantly different from low LDL cholesterol group at *$p<0.05$, †$p<0.01$, and ‡$p<0.001$. Values are given as age-adjusted mean±SEM.

TABLE 7. Partial Correlation Coefficient Between Dietary Intake and LDL Particle Score

Nutrient (g)	LDL particle score
Protein	−0.12
Carbohydrate	0.10
Total fat	−0.17*
Sat FA	−0.18*
Mono FA	−0.19†
Poly FA	−0.01
Animal fat	−0.21†
Cholesterol	−0.27‡

LDL, low density lipoprotein; FA, fatty acids. Analysis was carried out in a subset of 161 women and men, and values were adjusted for age, gender, and caloric intake.
*$p<0.05$, †$p<0.01$, ‡$p<0.001$.

LDL particles,[8,12] other studies report either no association[9,11] or a trend toward lower LDL cholesterol levels in subjects with small, dense LDL.[19,22] Our data indicate that the highest LDL cholesterol levels are found in subjects with LDL 3–5. Women with LDL 4 had significantly higher LDL cholesterol levels (mean, 148 mg/dl) than any other LDL type group, while the highest LDL cholesterol levels in men were found among those with LDL 3, 4, and 5 (mean, 138 mg/dl). Lower mean LDL cholesterol levels were found among subjects with LDL 1, 2, 5 (in women), 6, and 7. The parabolic association between LDL particle size and LDL cholesterol is of interest. In our view, human LDL particles 3–5 may be the most common LDL size observed in mildly hypercholesterolemic CAD patients because these LDL sizes are associated with the highest LDL to HDL cholesterol ratios compared with control subjects (4.15 versus 3.48, respectively).[22] In addition, LDL 1 and 2 were very rarely found among these male patients.[22] However, as shown in Figure 3, healthy Costa Rican men have LDL particle scores identical to those found in patients with CAD. In the absence of an increased LDL to HDL cholesterol ratio (2.78 in Costa Rican men),[16] these particles are less likely to be

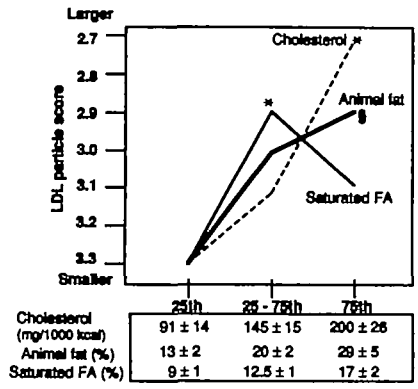

FIGURE 5. *Graph shows the mean low density lipoprotein (LDL) particle score for each dietary intake percentile. Table shows the mean±SD intakes of cholesterol, animal fat, and saturated fat (FA) in each percentile.* *$p<0.01$, §$p<0.05$ *indicates significantly different from LDL particle score in the lower 25th percentile, with values adjusted for age and gender (n=161).*

associated with atherosclerosis, provided that CAD mortality in Costa Rica is significantly lower than in the United States.[31]

LDL particle composition studies and epidemiological observations have shown that small, dense LDL is characterized by increasing protein content and reductions in cholesterol ester.[8,32,33] In addition, it has been demonstrated that LDL from patients with familial hypercholesterolemia contains more molecules of cholesterol ester than does LDL from normal individuals, even when the LDLs were matched for molecular weight.[34] In our study we found that when subjects with high-risk (≥160 mg/dl) and optimal (<130 mg/dl) LDL cholesterol levels were matched for LDL subclass groups, those in the high LDL cholesterol group had, on average, 11% higher LDL cholesterol to apo B ratios than those in the low LDL cholesterol group. The LDL cholesterol to apo B ratio decreased with decreasing size in both groups. Interestingly, women and men in the high LDL cholesterol groups were characterized by a decreased prevalence of LDL 1 and 2 and an increased prevalence of LDL 3 and 4. LDL heterogeneity has been associated with CAD in several studies.[19,22,34,35] While studies of monkeys have shown that the largest LDL particles are associated with atherosclerosis,[35] either buoyant LDL I[34,36] or small LDL III subclasses are frequently found in patients with CAD.[19,22] However, the association between LDL size and CAD is not independent of other established risk factors, especially LDL and HDL cholesterol levels.[22] Nevertheless, some properties of small, dense LDL per se, such as increased susceptibility to in vitro copper-induced oxidation, may increase their atherogenic potential.[37] Additionally, the presence of small, dense LDL particles could be a marker of lipoprotein alterations that predispose to CAD.[38]

An important factor to consider when examining LDL size as a risk factor for CAD in population studies is the effect of dietary intake on LDL particle size. Experiments in nonhuman primates have indicated that diets high in saturated fat and cholesterol cause progressive increases in LDL particle size because of an increase in cholesterol ester content.[39] These large particles are powerful predictors of atherosclerosis in monkeys.[40] In addition, isocaloric substitution of fish oil for lard is associated with smaller, n-3 fatty acid–enriched LDL particles, fewer cholesterol ester molecules, and lower transition temperatures.[41] Furthermore, we have previously reported that populations characterized by the consumption of low-fat diets have a higher prevalence of small, dense LDL particles and lower LDL cholesterol levels than those found in the United States.[16] Our data in this study support previous findings on the association between dietary intake and LDL particle size. Lower saturated fat and cholesterol intakes are associated with smaller, denser LDL particles within the Framingham population. Therefore, small LDL size per se is probably not a good indicator of atherosclerotic risk in population studies. Other environmental factors that affect LDL size, such as dietary intake, body habitus, physical activity, and use of medications, should be taken into consideration in population studies relating LDL size to CAD risk.[13–18,22]

The precise biochemical factors regulating LDL particle size are not completely understood. Clearly, an

important factor affecting LDL particle size is the plasma triglyceride level, as well as the efficiency of the lipolytic system as previously described.[42,43] In our study triglyceride levels alone explained 44% of the variance in LDL particle size. Decreased carbohydrate content of LDL protein and lipids, as well as a decreased LDL sialic acid content of apo B, has been associated with small LDL particles. These differences in the carbohydrate content of LDL subspecies may be associated with differential production of such particles or variability in their metabolic fate.[44] In addition, unusual LDL subclass profiles have been identified in patients with cholesteryl ester transfer protein deficiency, indicating that cholesteryl ester transfer protein activity may alter the metabolism of small LDL.[45] Lipoprotein and hepatic lipase activity may also be important determinants of LDL heterogeneity within and between genders. It has been hypothesized that hepatic lipase is involved in the conversion of intermediate density lipoprotein to LDL.[46] In fact, hepatic lipase deficiency appears to prevent LDL formation, resulting in the accumulation of large, buoyant, LDL-like particles.[47] Elevated hepatic lipase and decreased lipoprotein lipase activities have also been associated with the predominance of small, dense LDL in normal subjects.[48] Clearly, more needs to be learned about the precise factors regulating LDL particle subclasses.

In sum, LDL particle size distribution is skewed toward larger particles in women and is more symmetrical in men. The presence of smaller, secondary LDL peaks is associated with increased triglyceride levels, while LDL 3 or LDL 4 as a secondary peak is associated with higher LDL cholesterol levels. The current data are consistent with the concept that alterations in triglycerides and HDL cholesterol are strikingly associated with LDL particle size, so that individuals who have the largest LDL particles also have the lowest triglyceride and the highest HDL cholesterol levels. In addition, we report that individuals with the highest LDL cholesterol levels (≥160 mg/dl) have a higher prevalence of LDL types 3 and 4, as opposed to LDL 1 and 2, and an 11% higher LDL cholesterol to apo B ratio even when matched for LDL particle size. Furthermore, diets low in saturated fat and cholesterol are associated with smaller LDL particles. Therefore, the identification of small, dense LDL size per se may not be a good indicator of CAD risk in population studies. Our data indicate that LDL particle sizes 3–5 are associated with the highest LDL cholesterol levels.

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