

Lipoprotein Subclasses and Coronary Artery Calcium in Postmenopausal Women from the Healthy Women Study

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Lipoprotein subclass levels may improve the prediction of cardiovascular disease (CAD) in individuals beyond the risk assessment provided by conventional enzymatically determined lipid levels. The objective of this study was to evaluate the associations between nuclear magnetic resonance (NMR) spectroscopy-determined lipoprotein subclasses and coronary calcification in postmenopausal women, and to determine whether the associations were independent of conventional lipid measures. Coronary artery calcification (CAC) was measured by electron beam computed tomography, and lipoprotein subclasses were determined by NMR spectroscopy (Liposcience, Inc., Raleigh, NC), in 286 healthy women (mean age = 61.7), at 8 years postmenopause. CAC was analyzed as categories 0, 1 to 99, and ≥ 100 . The mean CAC was 53 (range, 0 to 1,175), and 54% of the women had 0 scores. Large high-density lipoprotein (HDL) was inversely associated with CAC category, but small HDL was not. All very low-density lipoprotein

(VLDL) subclasses—small, medium, and large—were positively associated with CAC ($p < 0.01$). Small low-density lipoprotein (LDL) was positively associated with CAC ($p < 0.01$), but medium and large LDL were not. Smaller LDL particle size ($p < 0.01$) and higher levels of LDL particles ($p < 0.001$) were associated with higher CAC category. In separate ordinal logistic regression models, small LDL, LDL particles, and large VLDL were each positively associated ($p < 0.05$) with higher CAC after adjustment for age, systolic blood pressure (SBP), current smoking, and conventional measures of LDL cholesterol, HDL cholesterol, and triglycerides. These results suggest that the measurement of lipoprotein subclasses may improve the prediction of CAD in postmenopausal women beyond that provided by the conventional lipid panel and CAD risk factors. ©2002 by Excerpta Medica, Inc.

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Coronary artery disease (CAD) is the leading cause of death in the United States for men and women.¹ One of the major advances in public health has been the development of pharmacological and nonpharmacological therapies for the primary and secondary prevention of clinical CAD.² However, because our ability to predict risk for an individual is limited, large numbers of people must be treated to prevent a single heart attack. The poor prediction of individual risk is especially problematic when evaluating men < 50 years, or women aged < 60 years, who have a relatively low 10-year risk of a heart attack (except for high-risk individuals with familial hypercholesterolemia or multiple risk factors, such as diabetes, smoking, hypertension, and high lipid levels).³ The majority of first heart attacks occur among individuals who are classified as moderate risk, as reported in fol-

low-up studies from the Multiple Risk Factor Intervention Trial (MRFIT)⁴ and the Framingham Study.⁵ In addition, the onset of a myocardial infarction or sudden CAD death is rapid, so that 60% to 70% of CAD deaths occur out of hospital. Even with improvement in emergency medical services, these CAD deaths are unlikely to be substantially reduced. Therefore, further reduction in CAD mortality will require reduction in the prevalence of the underlying, or subclinical, atherosclerotic disease in the population.

In the Cardiovascular Health Study, we have shown that indexes of subclinical atherosclerosis, which are prevalent among older men and women, are strong predictors of CAD.⁶ Other investigators have shown that the extent of coronary artery calcification (CAC), a noninvasive index of coronary atherosclerosis, is a strong predictor of the risk of myocardial infarction, even among younger men and women.⁷ Total and low-density lipoprotein (LDL) cholesterol are strongly associated with the development of clinical and subclinical atherosclerosis, but there is substantial interindividual variation in the extent of atherosclerosis, especially of the coronary arteries, at any level of total plasma cholesterol.⁸ Therefore, prevention of CAD will require not only a more aggressive effort to measure subclinical atherosclerosis in asymptomatic individuals, but also the improvement of the quantification of lipoproteins beyond conventional lipid levels. Noninvasive measures, such as carotid

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intimal-medial wall thickness, plaque, and, more recently, CAC, can be used to assess the extent of atherosclerosis. Nuclear magnetic resonance (NMR) spectroscopy of lipoproteins provides a promising new opportunity to quantify lipoproteins, including their subclasses, in large numbers of individuals, rapidly, and at relatively low cost.

High-density lipoprotein (HDL), LDL, and very low-density lipoprotein (VLDL) can be divided into subclasses based on size or density, and some subclasses appear to have stronger associations with CAD than others. In cross-sectional⁹⁻¹³ and prospective studies,¹⁴⁻¹⁶ small LDL size, large VLDL, small LDL, small HDL, and/or the number of LDL particles have been shown to be positively associated with atherosclerotic cardiovascular disease and myocardial infarction. These studies have generally used the time-consuming techniques of gradient gel electrophoresis or density-gradient ultracentrifugation to evaluate the lipoprotein subclasses. In addition, small LDL was not always significantly associated with CAD after adjustment for triglycerides, and few of the studies have included women. The NMR determination of lipoprotein subclasses has recently become commercially available (Liposcience, Inc., Raleigh, NC) and provides results that are reproducible and in agreement with measurements by other methods.^{17,18} In the Cardiovascular Health Study, we found that higher levels of NMR-determined small LDL and LDL particles predicted incident myocardial infarction and angina, independent of enzymatically determined HDL cholesterol and triglycerides, primarily among the elderly women.¹⁹ Our objective in the current study was to evaluate associations between NMR-determined lipoprotein subclasses and coronary calcification in healthy women who were approximately 8 years postmenopause, and to determine whether the associations were independent of conventional lipid measures.

METHODS

Between 1983 and 1985, 541 premenopausal women, aged 42 to 50 years, were enrolled in the Healthy Women Study (HWS), a longitudinal study of the effects of menopause on cardiovascular risk. The HWS measured standard demographic and cardiovascular risk factors, including triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol by conventional separation and enzymatic methods.²⁰ When the women were 8 years postmenopause, lipoprotein subclasses and coronary calcification were measured in addition to standard CAD risk factors. The number of women who were available (ie, who had reached their eighth postmenopausal year) and who consented to participate in this substudy was 286.

Determinations of lipids and lipoproteins: Blood samples were drawn after a 12-hour fast, and lipids were determined with conventional enzymatic methods.²⁰ Total cholesterol, HDL cholesterol, and triglycerides were measured by a lipid laboratory that uses Centers for Disease Control and Prevention (CDC) standards. LDL cholesterol was estimated using the Friedewald equation.²¹ Lipoprotein subclasses were

determined using NMR spectroscopy (Liposcience). The methodology has been described in detail.^{17,18} In brief, each lipoprotein subclass is quantified using the NMR signals, which differ in frequency and shape depending on the diameter of the lipoprotein particles. The individual signals are derived from the total recorded signal, using data from previously modeled reference lipoprotein subclasses. The intensity of each signal is proportional to the quantity of the subclass, which is multiplied by a standard lipid amount to provide the results in milligrams per deciliter of cholesterol for HDL and LDL, or milligrams per deciliter triglyceride for VLDL. The NMR spectroscopy method reported 6 VLDL subclasses, 4 LDL subclasses including IDL, and 5 HDL subclasses. For this analysis, the subclasses were combined into large (60–200 nm), medium (35–60 nm), and small (27–35 nm) VLDL; large (21.3–23.0 nm), medium (19.8–21.2 nm), and small (18.3–19.7 nm) LDL; and large (8.2–13 nm) and small (7.3–8.2 nm) HDL.

CAC scores: Electron beam computed tomography scans were conducted using an Imatron C-150 Scanner (Imatron, South San Francisco, CA). The heart was scanned from the aortic root to the apex in 30 to 40 contiguous 3-mm-thick transverse images. Calcification scores were calculated according to the Agatston method.²² The reproducibility of the electron beam computed tomography scans from this laboratory has been excellent, with an intraclass correlation of 0.99.

Statistical analyses: Coronary calcification scores were very skewed. Therefore, for analysis, we categorized CAC as 0, 1 to 99, and ≥ 100 . Analysis of covariance was used to determine age-adjusted mean levels of lipids and lipoprotein subclasses by coronary calcification category, with contrasts to test for linear trend. Ordinal logistic regression was used to determine whether the lipoprotein subclass measures were predictive of higher coronary calcification category after adjusting for enzymatically determined lipid levels.

RESULTS

Table 1 reports participant characteristics at the visit when the women were approximately 8 years postmenopause. The mean age was 61.7 years, with a range of 57 to 66 years, and 13% of the women were current smokers. In these postmenopausal women, the VLDL subclasses were evenly distributed between small, medium, and large particles. However, both LDL and HDL subclasses contained a higher proportion of large particles.

Table 1 also reports the age-adjusted means of the enzymatically determined lipid levels and the NMR-determined lipoprotein subclass levels for each CAC category and Spearman correlations between the risk factors and coronary calcification score. Similar to previous results from a smaller sample,²³ enzymatically determined LDL cholesterol and triglycerides were positively associated with CAC, and HDL cholesterol was inversely associated with CAC ($p < 0.05$

TABLE 1 Age-Adjusted Mean Risk Factors at 8 Years Postmenopause, by Coronary Artery Calcification (CAC) Categories, in 286 Women from the Healthy Women Study

	Mean ± SD Total Group (n = 286)	CAC Score			p for Linear Trend	Spearman Correlation
		0 (n = 154)	1–100 (n = 88)	(n = 44)		
Age (yr)	61.7 ± 1.7	61.7	61.7	61.8	0.711	0.02
Systolic BP, mm Hg	121.5 ± 17.3	119.6	123.4	124.2	0.134	0.12
Body-mass index	27.3 ± 5.3	26.5	28.7	27.6	0.251	0.09
Waist/hip ratio	0.79 ± 0.07	0.78	0.81	0.81	0.091	0.14*
Fasting glucose (mg/dL)	92.3 ± 17.9	89.0	94.5	99.1	0.002	0.18*
HDL-C† (mg/dL)	61.2 ± 17.3	63.9	58.7	56.8	0.021	−0.16*
LDL-C† (mg/dL)	127.7 ± 32.1	125.3	124.6	141.3	0.006	0.08
Total cholesterol† (mg/dL)	214.1 ± 34.5	212.2	209.1	228.9	0.007	0.06
Triglycerides† (mg/dL)	125.0 ± 73.3	114.8	129.1	152.2	0.005	0.15*
Small HDL (mg/dL)	18.1 ± 7.3	18.2	18.4	17.7	0.66	0.00
Large HDL (mg/dL)	42.5 ± 21.1	45	40.8	38.4	0.07	−0.10
HDL size (nm)	9.05 ± 0.50	9.11	8.96	8.95	0.049	−0.14*
Small LDL (mg/dL)	42.4 ± 47.9	34.4	47.9	60.6	0.001	0.16*
Medium LDL (mg/dL)	31.5 ± 37.7	31.8	34.5	25	0.29	−0.05
Large LDL (mg/dL)	87.5 ± 49.7	90.3	82.6	89.6	0.93	−0.05
LDL particles (nmol/L)	1,855 ± 558	1,755	1,912	2,094	0.001	0.20*
LDL size (nm)	20.95 ± 0.66	21.07	20.86	20.74	0.004	−0.15†
Small VLDL (mg/dL)	31.4 ± 18.8	29.1	32.1	38.6	0.003	0.17*
Medium VLDL (mg/dL)	38.2 ± 32.8	34.7	39.2	49.9	0.007	0.14*
Large VLDL (mg/dL)	24.9 ± 39.3	17.1	33.2	36.9	0.003	0.22*
VLDL size (nm)	47.0 ± 10.6	46.4	48.3	46.6	0.935	0.11

BP = blood pressure; HDL-C = high-density lipoprotein; LDL-C = low-density lipoprotein cholesterol; VLDL = very low-density lipoprotein.
 *p = ≤0.05.
 †Enzymatic determinations.

for all). NMR-determined small LDL increased with CAC category, but medium and large LDL did not. Mean LDL size decreased ($p < 0.001$) and LDL particles increased ($p = 0.004$) with higher CAC category. All of the VLDL subclasses were positively associated with CAC. Large HDL was inversely associated with coronary calcification, but small HDL was not. Figure 1 shows age-adjusted mean lipid and lipoprotein subclass levels for each category of coronary calcification, with enzymatically determined lipids on the left, and NMR-determined lipoprotein subclasses on the right.

We also examined Spearman correlations between the 3 LDL subclasses and CAD risk factors (Table 2). As expected, higher levels of small LDL correlated with larger waist circumference, higher fasting glucose, triglycerides, and LDL cholesterol, and lower HDL cholesterol. The correlations with medium LDL were similar, but weaker. In contrast, large LDL was inversely correlated with CAD risk factors and positively associated with HDL cholesterol.

Ordinal logistic regression was used to model the risk of being in a higher CAC category for 1 standard deviation (SD) increase in each lipoprotein subclass measure (Table 3). The odds ratios are adjusted for total HDL cholesterol, or for total LDL cholesterol and triglycerides. The protective effect of large HDL, with an odds ratio of 0.77, was no longer significant after adjusting for total HDL cholesterol. However, after adjusting for both LDL cholesterol and triglycerides, a 1 SD increase in small LDL made a postmenopausal woman in this study 36% more likely to be in a higher CAC category. After further adjustment

for HDL cholesterol, age, systolic blood pressure, and smoking, small LDL was still significantly associated with higher CAC. The same was true for higher LDL particle concentration and large VLDL. Smaller mean LDL size was significantly associated with higher CAC category after adjustment for LDL cholesterol and triglycerides but not after further adjustment for HDL cholesterol, age, smoking, and systolic blood pressure (SBP) (data not shown).

DISCUSSION

In these postmenopausal women, increased coronary calcification was associated with higher levels of small LDL and large VLDL, more LDL particles, smaller mean LDL size, and lower levels of large HDL. The associations with small LDL, LDL particles, and large VLDL remained significant after adjustment for LDL cholesterol, triglycerides, HDL cholesterol, age, smoking, and systolic blood pressure. These results suggest that prediction of cardiovascular risk in postmenopausal women may be improved by measuring lipoprotein subclasses in addition to conventional lipid levels.

For these postmenopausal women, small LDL, but not large LDL, was predictive of increased coronary calcification. Similar findings have been reported for men.^{13,24} Small LDL was also positively associated with CAD risk factors, such as higher triglycerides and larger waist circumference, whereas large LDL was inversely associated with those same risk factors. Most of the LDL cholesterol in these postmenopausal

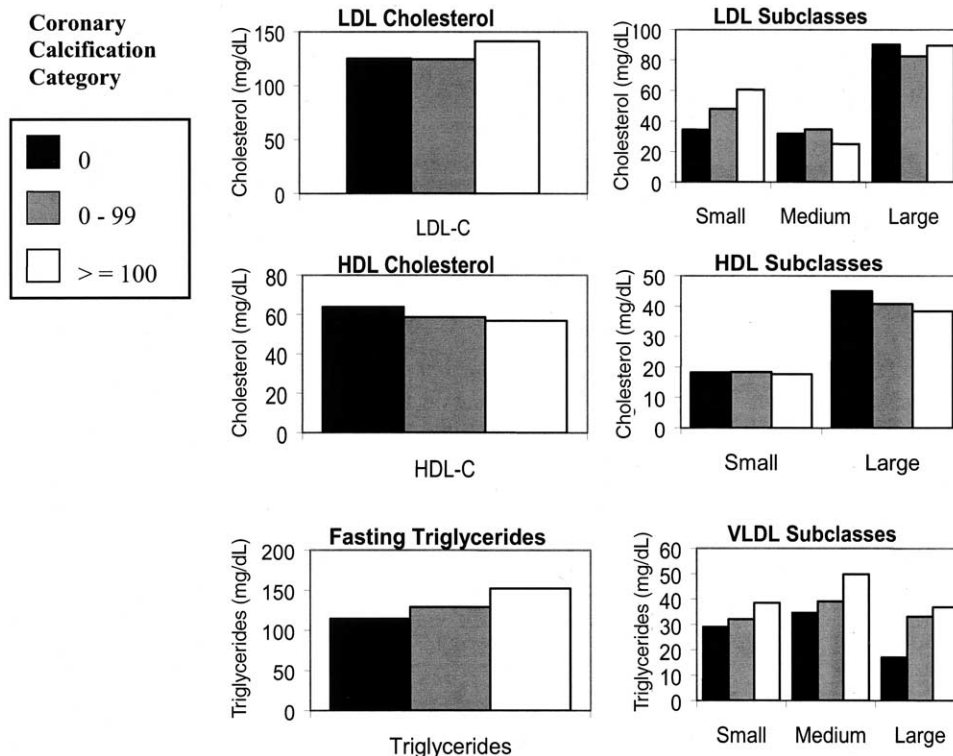


FIGURE 1. Mean enzymatically determined lipid and NMR-determined lipoprotein subclass levels by category of coronary calcification, adjusted for age. The p value is for linear trend from contrasts in analysis of covariance. HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; VLDL = very low-density lipoprotein.

TABLE 2 Spearman Correlations Between Low-Density Lipoprotein Subclasses and Cardiovascular Risk Factors in 286 Women at 8 Years Postmenopause: the Healthy Women Study

Risk Factor	Small LDL	Medium LDL	Large LDL
Waist circumference (cm)	0.19*	0.08	-0.24 [†]
Body mass index	0.09	0.10	-0.23 [†]
Fasting glucose (mg/dL)	0.13*	0.04	-0.21 [†]
Triglycerides [‡] (mg/dL)	0.27 [†]	0.13*	-0.36 [†]
LDL cholesterol [‡] (mg/dL)	0.13*	0.10	-0.25 [†]
HDL cholesterol [‡] (mg/dL)	-0.30 [†]	-0.15*	0.35 [†]
Total cholesterol [‡] (mg/dL)	0.08	0.04	-0.21 [†]

HDL = high-density lipoprotein; LDL = low-density lipoprotein.
 *p ≤ 0.05
 †p ≤ 0.001
 ‡Enzymatic determinations

women was contained in large LDL particles. These results support the hypothesis that in postmenopausal women, the risk associated with LDL cholesterol is primarily the result of small LDL particles.

The increased atherogenicity of small LDL particles in comparison to larger LDL particles may be the result of increased susceptibility to oxidation,²⁵ increased entry into the arterial wall, decreased affinity for LDL receptors,²⁶ and/or increased binding to intimal proteoglycans.²⁷ The Framingham Study has also found that the LDL subclass distribution was skewed toward large particles in women, whereas the LDL subclass distribution was more symmetrical in men.²⁸

Other studies have found that pattern B (in which small LDL particles predominate) is more common among men than among women. Differences between men and women in the LDL subclass distribution may explain women's lower CAD risk, despite similarities in LDL cholesterol levels between postmenopausal women and similar-aged men. Much of the epidemiology of cardiovascular disease among women may need to be reevaluated by including measures of the numbers and size of lipoprotein particles.

The levels of small LDL are also strongly and positively related to measures of the insulin resistance syndrome and diabetes mellitus and inversely related

TABLE 3 Odds of Being in a Higher Coronary Calcification Category for 1 Standard Deviation Increase in Lipoprotein Subclass, in 286 Postmenopausal Women: the Healthy Women Study

Variable	OR (95% CI)	Adjusted OR (95% CI)
Small HDL	0.98 (0.78–1.22)	0.85 (0.66–1.10)*
Large HDL	0.79 (0.63–0.99)	1.22 (0.82–1.83)*
Small LDL	1.47 (1.18–1.83)	1.36 (1.04–1.77)†
Medium LDL	0.95 (0.76–1.19)	0.78 (0.60–1.02)†
Large LDL	0.92 (0.74–1.16)	1.03 (0.77–1.39)†
LDL size	0.46 (0.28–0.73)	0.55 (0.31–0.99)†
LDL particles	1.57 (1.24–1.98)	1.44 (1.04–1.99)†
Small VLDL	1.38 (1.10–1.73)	1.22 (0.88–1.68)†
Medium VLDL	1.33 (1.06–1.65)	1.02 (0.75–1.39)†
Large VLDL	1.52 (1.20–1.91)	1.59 (1.11–2.28)†

CI = confidence interval; HDL = high-density lipoprotein; LDL = low-density lipoprotein; OR = odds ratio; VLDL = very low-density lipoprotein.
 *Adjusted for enzymatically determined HDL cholesterol.
 †Adjusted for enzymatically determined LDL cholesterol and triglycerides.

to HDL cholesterol levels.²⁹ The marked increase in risk of CAD for women with the insulin resistance syndrome and diabetes mellitus may be the result of higher levels of small, dense LDL and greater numbers of LDL particles. The efficacy of lipid-lowering therapy, especially statin drugs among diabetic men and women, was reinforced by the Heart Protection Study, reported at the 2001 American Heart Association meeting. This reported efficacy may be the result of the beneficial effect of statins in reducing the number of LDL particles.³⁰

The association between large VLDL and coronary calcification also persisted after adjustment for conventional lipid measures, including triglycerides, although it is not clear that large VLDL is directly atherogenic. The association with large VLDL may be the result of its role in lipoprotein metabolism. Triglyceride-rich VLDL provides the substrate for metabolic reactions, by means of cholesteryl ester transfer protein and hepatic lipase, which produce cholesterol-depleted LDL. Thus, the level of large VLDL triglyceride contributes to decreased LDL size and to increased LDL particles, at a given level of LDL cholesterol.

In summary, mean LDL size (diameter), number of LDL particles, and the distribution of particles among subclasses may provide more accurate risk indexes than conventional lipid levels, which measure the total amount of cholesterol or triglycerides carried by all of the particles in a major class (e.g., LDL). LDL subclass measurement may be especially important in postmenopausal women, for whom total LDL cholesterol measurements primarily reflect large LDL particles, which appear to be less atherogenic than small LDL particles. In these postmenopausal women, small LDL remained predictive of higher CAC after adjustment for CAD risk factors, including conventional lipids. Prospective studies are needed to determine whether small LDL predicts CAD events in women, whether it is a better predictor than conventional lipid levels, and whether the association differs by sex. Our results suggest that measuring lipoprotein subclass levels by NMR may improve cardiovascular risk as-

essment and pharmacological treatment in postmenopausal women.

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