Increased Small Low-Density Lipoprotein Particle Number

A Prominent Feature of the Metabolic Syndrome in the Framingham Heart Study

Sekar Kathiresan, MD; James D. Otvos, PhD; Lisa M. Sullivan, PhD; Michelle J. Keyes, MA; Ernst J. Schaefer, MD; Peter W.F. Wilson, MD; Ralph B. D'Agostino, PhD; Ramachandran S. Vasan, MD; Sander J. Robins, MD

Background—Levels of LDL cholesterol (LDL-C) are frequently not elevated in individuals with the metabolic syndrome (MetSyn). However, the atherogenic potential of LDL may depend on the number and size of LDL particles in addition to the cholesterol content of LDL.

Methods and Results—We examined the sex-specific cross-sectional relations of small LDL particle number (determined by nuclear magnetic resonance spectroscopy) to the presence of MetSyn and its components in 2993 Framingham Heart Study participants (mean age, 51 years; 53% women) without cardiovascular disease (CVD) and the relations of small LDL particle number to CVD incidence in people with MetSyn. The MetSyn (≥3 of 5 traits as defined by the National Cholesterol Education Adult Treatment Panel III) was present in 27% of men and 17% of women. In both sexes, small LDL particle number increased from 0 to 5 MetSyn traits, a pattern partly accounted for by strong correlations between small LDL particle number and serum triglycerides ($r=0.61$, $P<0.0001$) and HDL-C ($r=-0.55$, $P<0.0001$). Compared with participants without the MetSyn, those with the MetSyn had a higher CVD event rate. However, among participants with the MetSyn, CVD rates were similar for groups with an elevated versus a lower number of small LDL particles (defined by the sex-specific median).

Conclusions—Small LDL particle number is elevated in the MetSyn, increases with the number of MetSyn components, and most prominently is correlated with triglycerides and HDL-C. Whereas increased small LDL particle number identified the MetSyn with high sensitivity, a higher small LDL particle number was not associated with greater CVD event rates in people with the MetSyn. **(*Circulation*. 2006;113:20-29.)**

Key Words: cholesterol ■ lipids ■ lipoproteins ■ metabolic syndrome X ■ risk factors

The metabolic syndrome (MetSyn), a clustering of cardiovascular risk factors and metabolic abnormalities,[1,2] is known to be associated with an increased risk for cardiovascular disease (CVD).[3,4] It remains controversial whether the adverse risk associated with the MetSyn is greater than the sum of its parts.[5–8] However, clinical recognition of the MetSyn has been advocated by the National Cholesterol Education Program Adult Treatment Panel III (ATP III) as a means to focus on the clustering of commonly encountered CVD risk factors that often are a consequence of obesity.[9] ATP III defined the MetSyn by adverse levels of triglycerides, HDL cholesterol (HDL-C), blood pressure, glucose, and abdominal obesity.[10]

Clinical Perspective p 29

Persons with the MetSyn typically do not have elevated levels of LDL cholesterol (LDL-C). However, a qualitative abnormality in LDL—smaller LDL particle size—is recognized as being frequently associated with the atherogenic dyslipidemia that characterizes the MetSyn.[11] ATP III did not include LDL size among the clinical criteria for the MetSyn because of the uncertainty about the extent to which small LDL size predicts CVD risk independently of triglycerides and HDL-C (with which it is highly correlated).[9]

Recently, nuclear magnetic resonance (NMR) analysis of lipoproteins has been developed to directly quantify lipoprotein particle concentration for the several kinds of lipopro-

Received June 5, 2005; revision received September 16, 2005; accepted October 3, 2005.

From the National Heart, Lung, and Blood Institute's Framingham Heart Study (S.K., L.M.S., M.J.K., R.B.D., R.S.V., S.J.R.), Framingham, Mass; the Cardiology Division (S.K.), Massachusetts General Hospital, Harvard Medical School, Boston, Mass; the Broad Institute of Harvard and Massachusetts Institute of Technology (S.K.), Cambridge, Mass; LipoScience, Inc (J.D.O.), Raleigh, NC; the Statistics and Consulting Unit (L.M.S., M.J.K., R.B.D.), Department of Mathematics, Boston University, Boston, Mass; the Lipid Research Laboratory (E.J.S.), Tufts University School of Medicine, Boston, Mass; and the Department of Endocrinology, Diabetes, and Medical Genetics (P.W.F.W.), Medical University of South Carolina, Charleston.

Guest Editor for this article was Gregory L. Burke, MD, MSc.

Correspondence to Sander J. Robins, MD, Framingham Heart Study, 73 Mt Wayte Ave, Framingham, MA 01702. E-mail sjrobins@bu.edu

Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/CIRCULATIONAHA.105.567107**

teins that transport cholesterol in the blood, including small LDL particles.[12] Population-based information on NMR-measured small LDL particle number to help characterize the MetSyn is limited. Such information could help define the role of small LDL particle number as a potentially new key component in an expanded definition of the MetSyn, in keeping with a suggestion by a recent consensus conference.[13]

We sought to accomplish the following: (1) Define the relations between individual components of the MetSyn and small LDL particle number; (2) identify the lipid and nonlipid correlates of small LDL particle number; (3) assess the capacity of small LDL particle number as a single risk factor to predict the presence of the MetSyn; and (4) determine to what extent an increase in small LDL particles was associated with a greater risk of a new CVD event in participants with the MetSyn. Accordingly, we evaluated these questions in a community-based sample of nearly 3000 men and women free of clinical CVD.

Methods

Study Sample

The Framingham Heart Study offspring sample was recruited in 1971 with the inclusion of 5124 participants who were offspring and spouses of the original Framingham Heart Study cohort established in 1948.[14] From the 4019 attendees of the fourth offspring examination (1987 to 1990), 3511 had plasma available for NMR lipoprotein determination. We excluded participants for the following reasons: lipid-lowering treatment (n=136), fasting triglyceride concentration ≥400 mg/dL (n=66), prevalent CVD (n=246), and missing covariates (n=70). After exclusions, 2993 subjects (53% women) remained eligible. The institutional review board at Boston Medical Center approved the study, and all participants gave written, informed consent.

Clinical Evaluation

All participants underwent a routine medical history, physical examination including blood pressure measurement, anthropometry (body mass index and waist circumference), and laboratory assessment of CVD risk factors. Blood pressure was assessed as the average of 2 measurements taken after subjects had been seated for at least 5 minutes. Hypertension was defined as a systolic blood pressure ≥140 mm Hg, a diastolic blood pressure ≥90 mm Hg, or use of antihypertensive therapy. Diabetes was defined as a fasting plasma glucose value ≥126 mg/dL or use of hypoglycemic medications. CVD was defined by the presence of coronary heart disease (myocardial infarction, coronary insufficiency, and angina pectoris), death due to coronary heart disease, stroke, transient ischemic attack, intermittent claudication, or congestive heart failure. CVD diagnoses were determined by a panel of 3 physicians who reviewed all available medical and hospital records, as described previously.[15]

Participants were classified as having the MetSyn according to the 2004 revision of ATP III criteria.[10] By ATP III criteria, a person had the MetSyn when 3 or more of the following were present: (1) waist circumference >102 cm in men or >88 cm in women; (2) triglycerides ≥150 mg/dL; (3) HDL-C <40 mg/dL in men or <50 mg/dL in women; (4) systolic blood pressure ≥130 mm Hg, diastolic blood pressure ≥85 mm Hg, or use of antihypertensive therapy; and (5) fasting glucose ≥100 mg/dL.

Laboratory Methods

Plasma, with EDTA as an anticoagulant, was obtained from subjects who had fasted for at least 10 hours. Total cholesterol, triglycerides, and HDL-C were measured on an Abbott Diagnostics ABA-200 analyzer. LDL-C was estimated according to the Friedewald formula. Plasma concentrations of apolipoprotein B (apoB) were measured with a commercial immunoturbidimetric assay on a Spectrum CCX analyzer (Abbott Diagnostics) and reagents from Incstar Corp.[16] The interassay coefficients of variation were <5% for all lipids and <8% for apoB.

Lipoprotein subclass profiles were measured on plasma samples that had been stored at −70° by a commercially available proton NMR spectroscopic assay (LipoScience) as described previously.[17,18] In brief, with the characteristic signals broadcast by lipoprotein subclasses of different sizes, this NMR method directly measures the quantity of lipoprotein particles. Each subclass signal emanates from the total number of terminal methyl groups on the lipids contained within the lipoprotein particle core (cholesterol ester and triglycerides, each contributing 3 methyl groups) and in the lipoprotein particle shell (phospholipid and unesterified cholesterol, each contributing 2 methyl groups). The number of methyl groups contained within a particle depends, to a close approximation, only on the diameter of the lipoprotein particle and is not affected by differences in lipid composition; thus, the methyl NMR signal emitted by each subclass provides a direct measure of the concentration of that subclass.

The particle concentration of the following 9 lipoprotein species were determined: 3 VLDL subclasses [large, >60 nm (including chylomicrons); intermediate, 35 to 60 nm; small, 27 to 35 nm]; 3 LDL subclasses (IDL, 23 to 27 nm; large LDL, 21.3 to 23 nm; small LDL, 18.3 to 21.2 nm); and 3 HDL subclasses (large, 8.8 to 13 nm; intermediate, 8.2 to 8.8 nm; small, 7.3 to 8.2 nm). The small LDL

TABLE 1. Characteristics of the Study Sample

Variables	Men (n=1404)	Women (n=1589)
Clinical		
Age, y	51±10	51±10
SBP, mm Hg	129±17	124±19
DBP, mm Hg	82±10	77±10
Fasting glucose, mg/dL	97±26	92±22
Waist circumference, cm	97±11	81±13
Diabetes, %*	7	4
Hypertension, %	38	29
MetSyn criteria†		
SBP ≥130 mm Hg or DBP ≥80 mm Hg, %	55	40
HDL-C <50 mg/dL women or <40 mg/dL men, %	37	36
Waist circumference >35 in. in women or >40 in. in men, %	28	23
Triglycerides ≥150 mg/dL, %	29	16
Fasting glucose ≥100 mg/dL, %†	19	11
ATP III–defined MetSyn, %	27	17
Biochemical lipid measures		
LDL-C, mg/dL	134±33	127±36
HDL-C, mg/dL	44±12	56±15
Triglycerides, mg/dL	126±72	101±57
ApoB, mg/dL	102±23	94±24
NMR-derived lipoprotein measures		
LDL particle size, nm	20.7±0.6	21.1±0.4
LDL particle No., nmol/L	1526±409	1360±425

SBP indicates systolic blood pressure; DBP, diastolic blood pressure. Other abbreviations are as defined in text. Data shown are mean±SD or percentages.

*Includes treated diabetics.

†Criteria as defined in Grundy et al[10], the most recent revision of the ATP III.

TABLE 2. MetSyn Components and Lipoprotein Measures by Conventional Chemistry or NMR in Participants With and Without the MetSyn*

	Men			Women		
Variables	MetSyn+ (n=376)	MetSyn− (n=1028)	P	MetSyn+ (n=265)	MetSyn− (n=1324)	P
Age, y	54±0.5	50±0.3	<0.0001	55±0.6	50±0.3	<0.0001
ATP III MetSyn components						
SBP, mm Hg	135±0.8	126±0.5	<0.0001	136±1.0	121±0.4	<0.0001
DBP, mm Hg	86±0.5	80±0.3	<0.0001	83±0.6	75±0.3	<0.0001
Waist circumference, cm	106±0.5	94±0.3	<0.0001	98±0.7	78±0.3	<0.0001
Body mass index, kg/m^2	30.4±0.2	26.5±0.1	<0.0001	31.8±0.3	24.6±0.1	<0.0001
Fasting glucose, mg/dL	106±1	94±1	<0.0001	108±1	88±1	<0.0001
HDL-C, mg/dL	36±0.5	47±0.3	<0.0001	43±0.8	59±0.4	<0.0001
Triglycerides, mg/dL	192±3	102±2	<0.0001	171±3	87±1	<0.0001
NMR-derived lipoprotein measures						
Total LDL particle No., nmol/L	1718±20	1456±12	<0.0001	1624±24	1306±11	<0.0001
IDL, nmol/L	48±2	40±1	0.0006	61±3	34±1	<0.0001
Large LDL, nmol/L	388±15	608±9	<0.0001	563±18	711±8	<0.0001
Small LDL, nmol/L	1282±24	808±14	<0.0001	1001±23	561±10	<0.0001
Total VLDL particle No., nmol/L	97±2	72±1	<0.0001	91±2	64±1	<0.0001
Large VLDL, nmol/L	6±0.1	2±0.1	<0.0001	4±0.1	1±0.1	<0.0001
Intermediate VLDL, nmol/L	50±1	28±1	<0.0001	43±1	22±0.4	<0.0001
Small VLDL, nmol/L	41±1	43±1	0.18	44±1	42±1	0.09
Total HDL particle No., nmol/L	31±0.3	34±0.2	<0.0001	32±0.4	33±0.2	0.01
Large HDL, nmol/L	2±0.2	4±0.1	<0.0001	4±0.3	9±0.1	<0.0001
Intermediate HDL, nmol/L	8±0.2	7±0.1	0.0002	8±0.2	6±0.1	<0.0001
Small HDL, nmol/L	22±0.3	23±0.2	0.01	20±0.4	19±0.2	0.002
Lipoprotein particle size						
LDL size, nm	20.4±0.03	20.9±0.02	<0.0001	20.8±0.03	21.2±0.01	<0.0001
VLDL size, nm	54.2±0.4	44.8±0.2	<0.0001	51.8±0.4	42.0±0.2	<0.0001
HDL size, nm	8.7±0.02	9.0±0.01	<0.0001	9.0±0.03	9.5±0.01	<0.0001
Other measures						
LDL-C, mg/dL	136±2	134±1	0.16	136±2	125±1	<0.0001
ApoB, mg/dL	112±1	98±1	<0.0001	110±1	91±1	<0.0001

See the footnote to Table 1 and text for explanation of abbreviations. Values are mean±SE.
*All values were adjusted for age.

subclass comprises the sum of subclasses formerly labeled "intermediate" (19.8 to 21.2 nm) and "small" (18.3 to 19.7 nm),[17] because concentrations of both have very similar relations to lipid levels. Weighted-average VLDL, LDL, and HDL particle sizes (nm diameter) were computed as the sum of the diameter of each subclass multiplied by its relative mass percentage as estimated from the amplitude of its methyl NMR signal. Interassay coefficients of variation for NMR-derived lipoprotein particle concentrations were <3% for VLDL, LDL, and HDL; <4% for VLDL size; and <1% for LDL and HDL size. For all biochemical and NMR analyses, samples were handled in a blinded fashion such that all investigators had no knowledge of the participant's MetSyn status.

Statistical Analyses

Means and standard deviations are reported for continuous variables and proportions for categorical variables. All analyses were performed with SAS 8.[19] Sex-specific multiple linear-regression analysis was used to estimate differences between participants with and without the MetSyn after adjustment for age. To identify the NMR lipoprotein subclass features most significantly related to the MetSyn, we conducted sex-specific logistic regression with backward selection. The model consisted of MetSyn status as the dependent variable and the following candidate covariates: age, small LDL particle number, large LDL particle number, IDL particle number, total VLDL particle number, and total HDL particle number.

After adjustment for age and sex, partial Pearson correlations between small LDL particle number, MetSyn components, and apoB were calculated (PROC CORR). To address which component of the MetSyn was most strongly related to small LDL particle number, we used sex-specific linear-regression analysis (PROC REG) with small LDL particle number as the dependent variable and the following covariates: age, systolic blood pressure, diastolic blood pressure, waist circumference, fasting glucose, HDL-C, and triglycerides.

Tests of trend are presented to evaluate the association between age-adjusted NMR-derived lipoprotein or biochemical lipid measures and the number of MetSyn components. To address the question of whether apoB or small LDL particle number was more strongly associated with the MetSyn, we constructed logistic-regression models (PROC LOGISTIC) with the MetSyn as the dependent variable

TABLE 3. Correlations Among Small LDL Particle Number and Components of the MetSyn

	ApoB	SBP	DBP	Waist Circumference	Fasting Glucose	HDL-C	Triglycerides
Small LDL particle No.	0.61	0.19	0.20	0.30	0.20	−0.55	0.61
ApoB	...	0.18	0.20	0.28	0.16	−0.34	0.55
SBP	...	...	0.73	0.29	0.23	−0.06	0.23
DBP	...	...	...	0.32	0.17	−0.07	0.25
Waist circumference	...	...	...	...	0.28	−0.35	0.41
Fasting glucose	...	...	...	...	...	−0.14	0.18
HDL-C	...	...	...	...	...	...	−0.52
Triglycerides	...	...	...	...	...	...	...

See the footnote to Table 1 and text for explanation of abbreviations.
Data are Pearson partial correlations adjusted for age and sex.
P for all correlations <0.001.

and the following set of covariates: for model 1, age, sex, and small LDL particle number and for model 2, age, sex, and apoB.

To assess the utility of small LDL particle number to identify the MetSyn, we constructed sex-specific receiver operating characteristic (ROC) curves and calculated the areas under the curve. The area under the curve serves as an index of global test performance of small LDL particle number for identification of the MetSyn across the entire range of values, with an area under the curve of 0.5 indicating no discrimination ability.[20] We evaluated the sensitivity, specificity, positive predictive value, and negative predictive value for select LDL particle number cutpoints for identifying the MetSyn.

To assess whether CVD incidence among those with the MetSyn varied according to small LDL particle number or LDL-C, we calculated age-adjusted CVD event rates for 3 strata using Cox proportional hazards model: MetSyn absent, MetSyn present/below sex-specific median of small LDL particle number (for the group with MetSyn), and MetSyn present/above sex-specific median of small LDL particle number (for the group with the MetSyn). These analyses were sex specific. Similar strata were constructed by substituting LDL-C for small LDL particle number. CVD events occurring from the time of baseline examination (cycle 4, 1987 to 1990) to December 2004 were included in the analysis. Person-years of follow-up were accrued from baseline to the date of the first event or censored at the date of December 2004 if free of a CVD event.

Because we investigated 18 biochemical and NMR-derived lipid/lipoprotein measures, a 2-sided $P<0.003$ was considered statistically significant. This significance criterion is based on the Bonferroni correction to account for multiple testing.

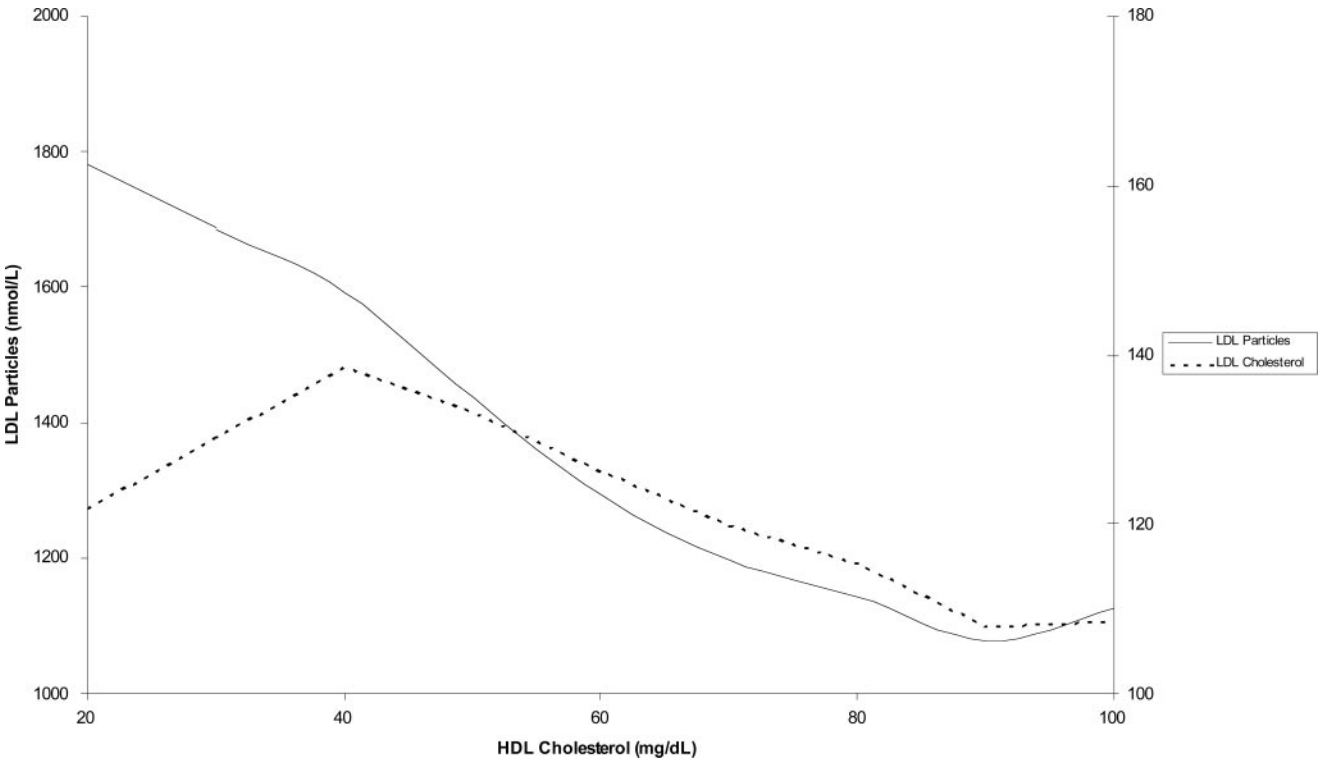

Figure 1. Relations of total LDL particle number (nmol/L) and LDL cholesterol value (mg/dL) to the level of HDL cholesterol (mg/dL). Curves represent mean age-adjusted LDL particle number and LDL cholesterol values in Framingham Offspring Study participants (n=2993) grouped into 10-mg/dL increments of HDL cholesterol.

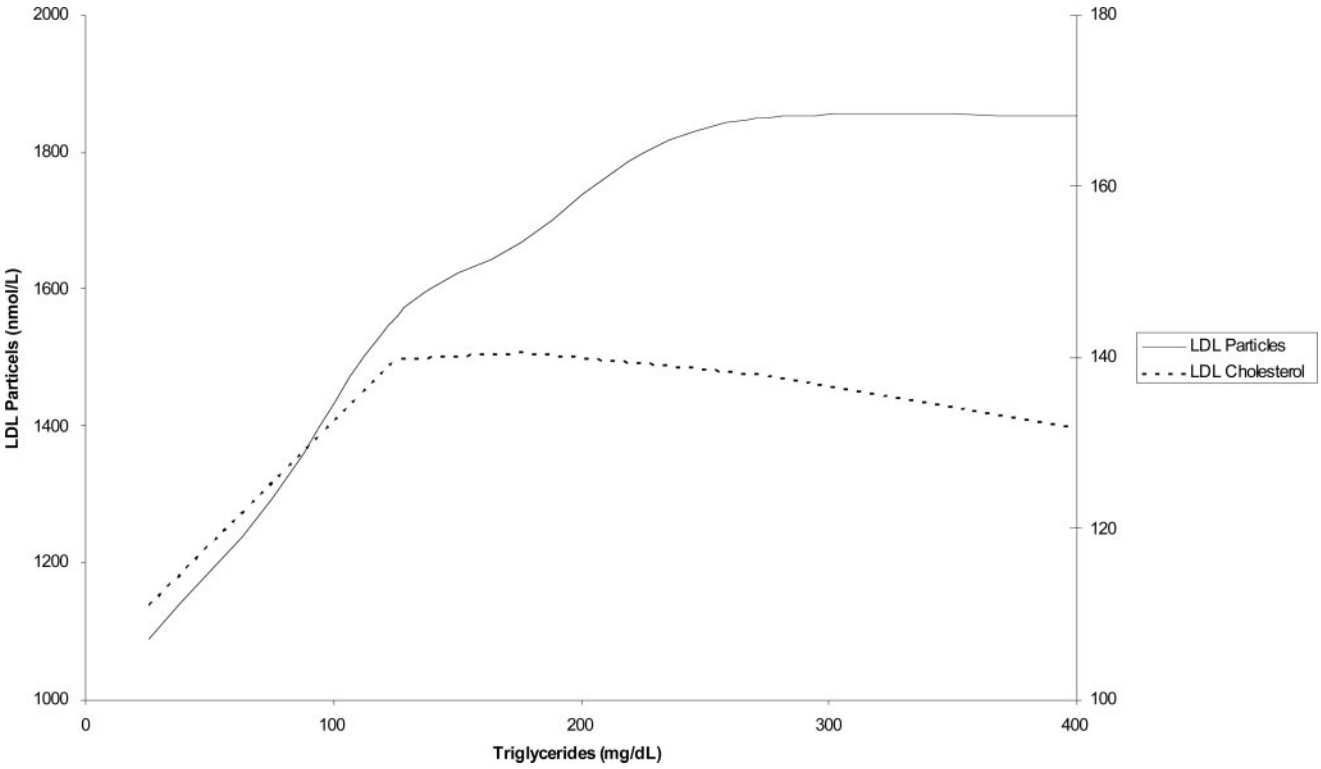

Figure 2. Relations of total LDL particle number (nmol/L) and LDL cholesterol value (mg/dL) to the level of triglycerides (mg/dL). Curves represent mean age-adjusted LDL particle number and LDL cholesterol values in Framingham Offspring Study participants (n=2993) grouped into 25-mg/dL increments of triglyceride level.

Results

The characteristics of our study sample are shown in Table 1. The mean age of this population was 51 years, and 53% were women. Hypertension was present in more than a third of the sample. MetSyn was present in 27% of the men and 17% of the women.

Plasma levels of lipids and apoB in men and women with or without the MetSyn are displayed in Table 2. As would be expected, all laboratory and clinical parameters that were used to define the presence of the MetSyn were significantly different from those in participants without the MetSyn. In addition, apoB was higher in those with the MetSyn than in those without the MetSyn. In contrast, mean levels of LDL-C were similar in men with or without the MetSyn, although in women with the MetSyn, LDL-C was modestly higher than in those without the MetSyn.

Compared with those without the MetSyn, participants with the MetSyn had significantly higher small LDL particle number (Table 2). Mean LDL particle size was smaller in the MetSyn as a result of the change in LDL subclass distribution to predominantly smaller LDL particles (Table 2). The NMR lipoprotein particle measures show that the higher triglyceride levels of participants with the MetSyn arose predominantly from increased numbers of large and intermediate-size VLDL subclasses, not from differences in small VLDL particle concentrations (Table 2). Similarly, the lower HDL-C levels associated with the MetSyn resulted mainly from fewer numbers of large HDL subclass particles.

Given that participants with and without the MetSyn differed across a number of lipoprotein subclass measures, we sought to identify the most prominent differences through backward selection. In both men and women, small LDL particle number, total VLDL particle number, and total HDL particle number were each significantly related to MetSyn (each $P<0.0001$) in the final stepwise model.

Small LDL particle number was significantly correlated with each component of the MetSyn, with the strongest correlations involving HDL-C and triglycerides (Table 3). Small LDL particle number was also strongly correlated with serum apoB (Table 3). In sex-specific linear-regression models with small LDL particle number as the dependent variable and the 5 MetSyn components as independent variables, we identified HDL-C and triglycerides as the only 2 components significantly associated with small LDL particle number (each $P<0.0001$).

The variation in total LDL particle number and LDL-C levels as a function of HDL-C concentration is displayed in Figure 1. As HDL-C level decreased, total LDL particle number continually rose, whereas the level of LDL-C remained relatively low (<140 mg/dL). A similar pattern was observed when we compared levels of total LDL particles and LDL-C as a function of triglyceride level (Figure 2). As the triglyceride level increased to ≈300 mg/dL, the number of total LDL particles rose while the levels of LDL-C again remained low.

Small LDL particle number and total LDL particle number each increased with the number of MetSyn components in both sexes, whereas the number of large LDL particles decreased (Table 4). ApoB concentrations were also associated with the number of MetSyn components in both men and

TABLE 4. Plasma Levels of NMR-Determined Lipoprotein Measures and Biochemical Lipid Measures With Increasing Number of MetSyn Features*

	No. of Components of MetSyn						
	0	1	2	3	4	5	*P* for Trend
Women	n=562	n=464	n=298	n=134	n=102	n=29	
NMR-derived lipoprotein measures							
Total LDL particle No., nmol/L	1169±16	1344±17	1496±22	1600±32	1678±37	1663±69	<0.0001
Small LDL particles, nmol/L	428±15	591±16	756±20	918±30	1090±34	1187±64	<0.0001
Large LDL particles, nmol/L	714±12	716±13	697±17	618±25	529±28	419±53	<0.0001
Biochemical lipid measures							
LDL-C, mg/dL	117±1	128±2	135±2	137±3	138±3	133±6	<0.0001
ApoB, mg/dL	84±1	92±1	101±1	110±2	111±2	113±4	<0.0001
Triglycerides, mg/dL	71±2	84±2	121±2	154±4	188±4	211±8	<0.0001
HDL-C, mg/dL	66±1	57±1	51±1	45±1	40±1	36±2	<0.0001
Men	n=286	n=407	n=335	n=233	n=113	n=30	
NMR-derived lipoprotein measures							
Total LDL particle No., nmol/L	1290±23	1485±19	1554±21	1690±25	1783±36	1767±69	<0.0001
Small LDL particles, nmol/L	574±26	813±21	991±24	1232±29	1396±41	1361±79	<0.0001
Large LDL particles, nmol/L	684±17	630±14	520±16	411±19	336±27	362±52	<0.0001
Biochemical lipid measures							
LDL-C, mg/dL	127±2	137±2	135±2	137±2	135±3	136±6	0.01
ApoB, mg/dL	90±1	99±1	103±1	111±1	115±2	115±4	<0.0001
Triglycerides, mg/dL	71±3	96±3	133±3	178±4	214±5	231±10	<0.0001
HDL-C, mg/dL	52±1	48±1	43±1	37±1	33±1	32±2	<0.0001

See the footnote to Table 1 and text for explanation of abbreviations.
*All values are expressed as mean±SE and have been adjusted for age.

women. The association between small LDL particle number and the MetSyn was modestly stronger than that between apoB and the MetSyn; the *c* statistic associated with an age- and sex-adjusted model including small LDL particle number was 0.81, and for that including apoB, it was 0.75.

We evaluated the capacity of small LDL particle number to differentiate the presence from the absence of the MetSyn by an ROC curve analysis (Figure 3). In women, the area under the ROC curve was 0.82 (95% confidence interval, 0.80 to 0.85). In women, a small LDL particle number cutoff value of 800 nmol/L had a sensitivity of 62% and a specificity of 84% for diagnosis of the MetSyn (Table 5). Similar results were seen in men (Figure 3).

Among participants with the MetSyn, we evaluated whether small LDL particle number (or LDL-C) was associated with an increased risk of incident CVD (Table 6). Compared with participants without the MetSyn, participants with the MetSyn had a higher rate of CVD events; however, among men and women with the MetSyn, CVD event rates did not differ significantly between those with a small LDL particle number above versus below the median (Table 6). Similarly, among men and women with the MetSyn, CVD event rates did not differ significantly between those with an LDL-C above or below the median (Table 7).

Discussion

Principal Findings

In the Framingham Heart Study, a community-based longitudinal study, the MetSyn as defined by ATP III was present in 27% of men and 17% of women at an average age of 51 years. Both men and women with the MetSyn had a higher number of small LDL particles; mean small LDL particle number was essentially proportional to the number of MetSyn components present. This pattern was probably in part accounted for by strong correlations between small LDL particle number, serum triglycerides, and HDL-C. In contrast to the progressive increase in small LDL particle number with increasing components of the MetSyn, LDL-C concentrations remained relatively stable. Small LDL particle number alone was modestly sensitive and specific for the diagnosis of MetSyn. Whereas individuals with the MetSyn had elevated small LDL particle number, CVD rates were similar for groups with an elevated versus a lower number of small LDL particles (defined by the sex-specific median).

Previous Studies

Prior work based on gradient gel electrophoresis has demonstrated that individuals with the MetSyn have smaller, more dense LDL particles.[21] Furthermore, with gel electrophoresis, small, dense LDL particles have also been associated with increased triglycerides, lower HDL-C, and increased apoB levels.[11,22,23] Although LDL particle numbers cannot be directly assessed by gel electrophoresis, apoB does provide a measure of combined LDL and triglyceride-rich lipoprotein (mainly VLDL) particle numbers[24] and is a good surrogate measure for increased LDL particle numbers in people with diabetic dyslipidemia[24] as well as with the MetSyn and insulin resistance.[25,26]

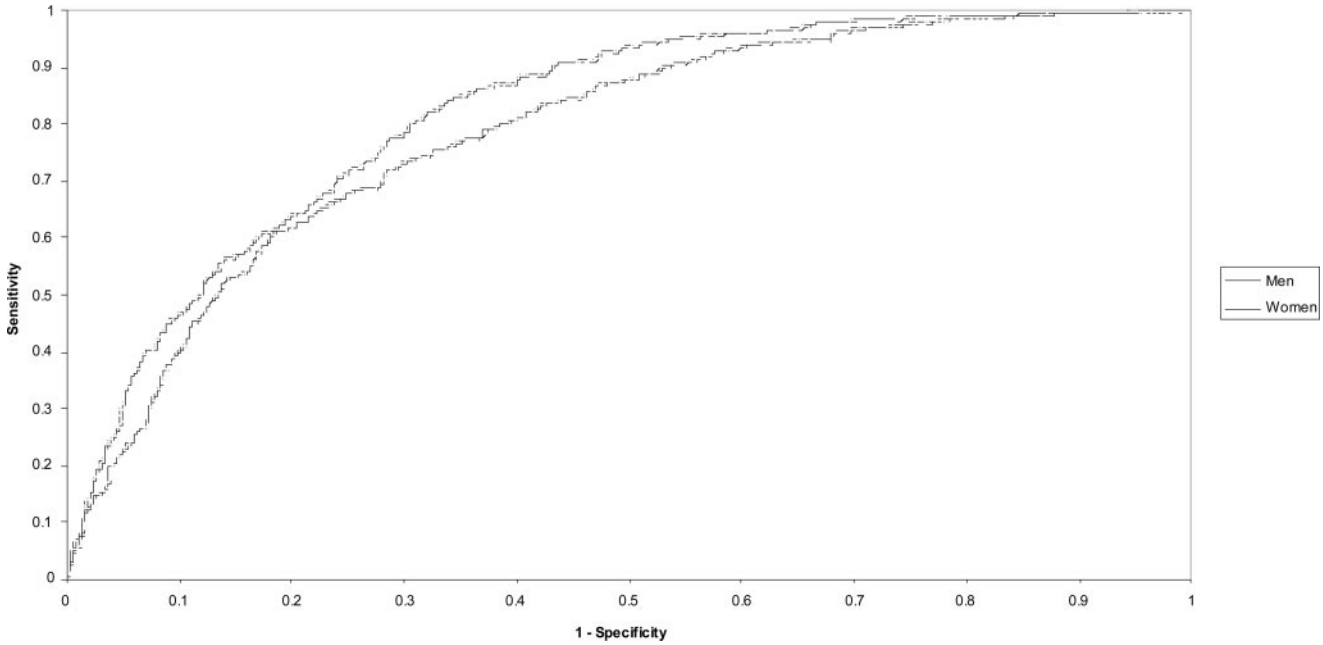

Figure 3. ROC curve for various cutoff levels for small LDL particle number in differentiating participants with the MetSyn from those without. Abbreviations are as defined in text.

Compared with apoB measurement, a more detailed assessment of LDL particle numbers and size can be achieved with NMR spectroscopy. Two prior studies have evaluated the relations between NMR-measured LDL particle number and the risk of CVD. In a nested case-control study of 260 women, Blake et al[27] found that the number of small LDL particles was a predictor of CVD risk. In a sample of elderly individuals assessed in a similar nested case-control design, Kuller et al[28] found that the number of small LDL particles predicted CVD risk in women but not in men.

LDL-C and LDL Particle Number

The present study illustrates and provides a potential explanation for the dissociation between LDL-C and LDL particle number in individuals with the MetSyn. We show that the increase in small LDL particle numbers seen in the MetSyn is

TABLE 5. ROCs for Various Cutoff Levels for Small LDL Particle Number in Differentiating Participants With and Without the MetSyn

Small LDL Particle No., nmol/L	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predictive Value	Negative Predictive Value
Men*				
800	85 (81, 88)	59 (56, 62)	43 (39, 46)	91 (89, 93)
900	75 (71, 79)	65 (62, 68)	44 (40, 48)	88 (85, 90)
1000	70 (66, 75)	71 (69, 74)	47 (43, 51)	87 (84, 89)
1100	61 (56, 66)	78 (75, 80)	50 (45, 55)	84 (82, 87)
1200	55 (49, 60)	82 (80, 84)	52 (47, 57)	83 (81, 85)
1300	47 (42, 52)	85 (83, 87)	53 (48, 59)	81 (79, 84)
Women†				
800	62 (56, 67)	84 (82, 86)	43 (38, 48)	92 (90, 93)
900	52 (46, 58)	88 (86, 90)	46 (41, 52)	90 (88, 92)
1000	43 (37, 49)	91 (90, 93)	49 (42, 55)	89 (87, 90)
1100	38 (32, 44)	94 (92, 95)	54 (47, 61)	88 (87, 90)
1200	31 (25, 37)	95 (94, 96)	56 (48, 64)	87 (86, 89)
1300	24 (19, 29)	96 (95, 97)	53 (44, 62)	86 (85, 88)

CI indicates confidence interval. See the footnote to Table 1 and text for explanation of other abbreviations.

*Area under the ROC curve, 0.79 (95% CI, 0.76–0.81).

†Area under the ROC curve, 0.82 (95% CI, 0.80–0.85).

TABLE 6. Age-Adjusted Incidence of CVD in Relation to MetSyn Status and Small LDL Particle Number

	Men		Women	
	n CVD/n at Risk†	CVD Event Rate per 1000 Person-Years (95% CI)	n CVD/n at Risk‡	CVD Event Rate per 1000 Person-Years (95% CI)
Without MetSyn	86/1028	77 (60, 94)	56/1324	40 (28, 51)
With MetSyn and below median small LDL particle No.*	43/188	161 (111, 211)	13/133	80 (36, 124)
With MetSyn and at or above median small LDL particle No.*	43/188	190 (135, 244)	17/132	89 (45, 134)

CI indicates confidence interval. See the footnote to Table 1 and text for explanation of other abbreviations.

*Median small LDL particle number in men with the MetSyn was 1257 nmol/L and in women with the MetSyn, 922 nmol/L.

†*P* value comparison among men with the MetSyn and below median small LDL particle number with those at or above median small LDL particle number is 0.55.

‡*P* value comparison among women with the MetSyn and below median small LDL particle number with those at or above median small LDL particle number is 0.84.

coupled to a significant reduction in the numbers of large LDL particles. This decrease in large LDL particle number is not numerically equivalent to the increase in small LDL particle number, and so, with a transition to more, smaller LDL particles, total LDL particle number is increased in the MetSyn. However, because large LDL particles contain much more cholesterol than do small LDL particles, the decrease in large LDL particles may not result in a change in the total LDL-C concentration. Thus, whereas LDL-C may not be much different in those with and without the MetSyn, LDL particle number and in particular, small LDL particle number may differ dramatically.

Mechanism Linking Elevated Small LDL Particle Number and the MetSyn

We observed that increases in the numbers of small LDL particles clustered with the MetSyn and that this association was closely linked to increases in triglycerides (or NMR-measured VLDL particle numbers) and decreases in HDL-C (or NMR-measured HDL particle numbers). These close associations would seem to be due to the strong relation of the MetSyn to underlying insulin resistance.[29] Several investigators have demonstrated that insulin resistance is associated with increased small LDL particle numbers. In the Insulin Resistance Atherosclerosis Study, Goff et al[30] reported that insulin resistance was correlated with increased numbers of small LDL particles by NMR analysis. Very similar results were reported by Garvey and associates,[31] who found that when compared with insulin-sensitive subjects, those with insulin resistance and diabetes had increased numbers of small LDL particles and higher total LDL particle numbers.

How might the MetSyn with underlying insulin resistance lead to the generation of increased numbers of small LDL particles? There is substantial evidence to show that excess adiposity, especially in the presence of diabetes or insulin resistance, leads to increased free fatty acid production by adipocytes.[24,32] These free fatty acids are taken up by the liver and used for new triglyceride synthesis. As a consequence of increased hepatic triglyceride synthesis, there is increased synthesis and secretion of VLDL particles by the liver.[33] These VLDL particles represent the substrate for 2 sequential reactions that could lead to an increased number of small LDL particles in the circulation. First, through the action of cholesterol ester transfer protein, an appreciable amount of triglyceride in VLDL may be exchanged for cholesterol ester in plasma LDL.[34] Second, these now-triglyceride-enriched (and cholesterol-depleted) LDL particles become a favorable substrate for hepatic lipase and may be transformed into smaller LDL particles by lipase-mediated triglyceride hydrolysis.[35] The metabolic model described seems best to integrate the steady-state and dynamic lipoprotein studies available to date (reviewed in Berneis and Krauss[36] and Packard[37]).

TABLE 7. Age-Adjusted Incidence of CVD in Relation to MetSyn Status and LDL-C

	Men		Women	
	n CVD/n at Risk†	CVD Event Rate per 1000 Person-Years (95% CI)	n CVD/n at Risk‡	CVD Event Rate per 1000 Person-Years (95% CI)
Without MetSyn	86/1028	77 (60, 94)	56/1324	39 (28, 51)
With MetSyn and below median LDL-C*	41/188	155 (106, 203)	15/132	89 (43, 135)
With MetSyn and at or above median LDL-C*	45/188	196 (141, 252)	15/133	81 (38, 123)

CI indicates confidence interval. See the footnote to Table 1 and text for explanation of other abbreviations.

*Median LDL-C in men with the MetSyn was 136 mg/dL and in women with the MetSyn, 136 mg/dL.

†*P* value comparison among men with the MetSyn and below median LDL-C with those at or above median LDL-C is 0.29.

‡*P* value comparison among women with the MetSyn and below median LDL-C with those at or above median LDL-C is 0.71.

Small LDL Particle Number and CVD

Small LDL particles have been suggested to be inherently more atherogenic, binding less well to the LDL receptor,[38] entering the arterial wall more easily[39,40] and showing greater susceptibility to oxidation than large LDL.[41] As a result, it has been hypothesized that the CVD risk associated with the MetSyn may at least in part be attributable to small LDL particles;[36,42] however, this hypothesis has been challenged.[43]

We separated those with the MetSyn into groups with high and low numbers of small LDL particles and did not find that higher numbers of small LDL particles were associated with greater CVD risk. Several explanations for this observation are possible. We evaluated the discriminative power of small LDL particle number only in a high-risk subset, those with the MetSyn. It is possible that small LDL particle number will better predict CVD risk across a broader spectrum of subjects that also includes individuals without the MetSyn. Alternatively, small LDL particle number elevation may be related to clinical CVD through the correlated changes in several other potential proatherogenic features (ie, high triglycerides, low HDL-C, increased apoB).[43] In this context, small LDL particle number may serve as marker of a metabolic condition and not necessarily directly promote atherosclerosis.[44]

Study Limitations

Several potential limitations of this analysis deserve mention. First, the Framingham Heart Study population is predominantly white, and consequently, our findings may not be entirely applicable to other populations. Second, the CVD event rates that were tabulated and related to the presence or absence of the MetSyn and to numbers of small LDL particles represent a variety of vascular pathological processes. Consequently, it is possible that high small LDL particle numbers may be related to a single or a more restricted spectrum of CVD events than was used for this analysis. Third, the Framingham population selected for this analysis was relatively young (averaging 51 years) with probably fewer individuals with the MetSyn than if this population had been older. Last, insufficient statistical power may have limited our ability to detect a relation between small LDL particle number and CVD risk in the MetSyn, and studies in larger samples may be needed.

Conclusion

An increase in LDL particle numbers, especially small LDL, parallels an increase in MetSyn components in both men and women in the Framingham Heart Study population. The increase in small LDL particles was especially associated with increases in triglycerides and decreases in HDL-C but was not reflected by changes in the concentration of LDL-C. Although increased small LDL particle number as a single measure is highly predictive of the MetSyn, a higher small LDL particle number was not associated with an increased CVD event rate in people with the MetSyn.

Acknowledgments

This work was supported through National Institutes of Health/National Heart, Lung, and Blood Institute contract N01-HC-25195 and 2K24 HL04334 (Dr Vasan). Dr Kathiresan is supported by an ACC Foundation/Merck Adult Cardiology Research Fellowship Award and a GlaxoSmithKline Research and Education Foundation for Cardiovascular Disease's Young Investigator Award.

Disclosure

Dr Otvos is an employee of LipoScience, Inc.

References

1. Reaven GM. Banting Lecture 1988: role of insulin resistance in human disease. *Diabetes*. 1988;37:1595–1607.
2. Ferrannini E, Haffner SM, Mitchell BD, Stern MP. Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. *Diabetologia*. 1991;34:416–422.
3. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*. 2001;24:683–689.
4. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA*. 2002;288:2709–2716.
5. Kahn R, Buse J, Ferrannini E, Stern M. The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care*. 2005;28:2289–2304.
6. McNeill AM, Rosamond WD, Girman CJ, Golden SH, Schmidt MI, East HE, Ballantyne CM, Heiss G. The metabolic syndrome and 11-year risk of incident cardiovascular disease in the Atherosclerosis Risk in Communities Study. *Diabetes Care*. 2005;28:385–390.
7. Ford ES. Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence. *Diabetes Care*. 2005;28:1769–1778.
8. Dekker JM, Girman C, Rhodes T, Nijpels G, Stehouwer CDA, Bouter LM, Heine RJ. Metabolic syndrome and 10-year cardiovascular disease risk in the Hoorn Study. *Circulation*. 2005;112:666–673.
9. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*. 2001;285:2486–2497.
10. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation*. 2004;109:433–438.
11. Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype: a proposed genetic marker for coronary heart disease risk. *Circulation*. 1990;82:495–506.
12. Otvos JD, Jeyarajah EJ, Bennett DW. Quantification of plasma lipoproteins by proton nuclear magnetic resonance spectroscopy. *Clin Chem*. 1991;37:377–386.
13. International Diabetes Federation. The IDF Consensus worldwide definition of the metabolic syndrome [article online]. Available at www.idf.org. Last accessed September 12, 2005.
14. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families: the Framingham Offspring Study. *Am J Epidemiol*. 1979;110:281–290.
15. Kannel WB, Wolf P, Garrison RJe. The Framingham Study: an epidemiological investigation of cardiovascular disease, section 34: some risk factors related to the annual incidence of cardiovascular disease and death in pooled repeated biennial measurements: Framingham Heart Study, 30-year follow-up. Bethesda, Md: National Heart, Lung, and Blood Institute; NIH publication No. 87-2703). 1987.
16. Contois JH, McNamara JR, Lammi-Keefe CJ, Wilson PW, Massov T, Schaefer EJ. Reference intervals for plasma apolipoprotein B determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. *Clin Chem*. 1996;42:515–523.
17. Freedman DS, Otvos JD, Jeyarajah EJ, Shalaurova I, Cupples LA, Parise H, D'Agostino RB, Wilson PW, Schaefer EJ. Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. *Clin Chem*. 2004;50:1189–1200.
18. Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. *Clin Lab*. 2002;48:171–180.
19. *SAS/STAT User's Guide, Version 8.1*. Cary, NC: SAS Institute, Inc; 2000.
20. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem*. 1993;39:561–577.

21. Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. *J Clin Invest*. 1993;92:141–146.
22. McNamara JR, Campos H, Ordovas JM, Peterson J, Wilson PW, Schaefer EJ. Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution: results from the Framingham Offspring Study. *Arteriosclerosis*. 1987;7:483–490.
23. Swinkels DW, Demacker PN, Hendriks JC, van 't Laar A. Low density lipoprotein subfractions and relationship to other risk factors for coronary artery disease in healthy individuals. *Arteriosclerosis.* 1989;9:604–613.
24. Sniderman AD, Scantlebury T, Cianflone K. Hypertriglyceridemic hyperapob: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. *Ann Intern Med*. 2001;135:447–459.
25. Sung KC, Hwang ST. Association between insulin resistance and apolipoprotein B in normoglycemic Koreans. *Atherosclerosis*. 2005;180:161–169.
26. Williams K, Sniderman AD, Sattar N, D'Agostino R Jr, Wagenknecht LE, Haffner SM. Comparison of the associations of apolipoprotein B and low-density lipoprotein cholesterol with other cardiovascular risk factors in the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation*. 2003;108:2312–2316.
27. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. *Circulation*. 2002;106:1930–1937.
28. Kuller L, Arnold A, Tracy R, Otvos J, Burke G, Psaty B, Siscovick D, Freedman DS, Kronmal R. Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol*. 2002;22:1175–1180.
29. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet*. 2005;365:1415–1428.
30. Goff DC Jr, D'Agostino RB Jr, Haffner SM, Otvos JD. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations: results from the Insulin Resistance Atherosclerosis Study. *Metabolism*. 2005;54:264–270.
31. Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, Pugh K, Jenkins AJ, Klein RL, Liao Y. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes*. 2003;52:453–462.
32. Sniderman AD, Cianflone K. Substrate delivery as a determinant of hepatic apoB secretion. *Arterioscler Thromb*. 1993;13:629–636.
33. Lewis GF, Uffelman KD, Szeto LW, Weller B, Steiner G. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J Clin Invest*. 1995;95:158–166.
34. Halle M, Berg A, Baumstark MW, Konig D, Huonker M, Keul J. Influence of mild to moderately elevated triglycerides on low density lipoprotein subfraction concentration and composition in healthy men with low high density lipoprotein cholesterol levels. *Atherosclerosis*. 1999;143:185–192.
35. Watson TD, Caslake MJ, Freeman DJ, Griffin BA, Hinnie J, Packard CJ, Shepherd J. Determinants of LDL subfraction distribution and concentrations in young normolipidemic subjects. *Arterioscler Thromb*. 1994;14:902–910.
36. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. *J Lipid Res*. 2002;43:1363–1379.
37. Packard CJ. Triacylglycerol-rich lipoproteins and the generation of small, dense low-density lipoprotein. *Biochem Soc Trans*. 2003;31:1066–1069.
38. Nigon F, Lesnik P, Rouis M, Chapman MJ. Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with the cellular LDL receptor. *J Lipid Res*. 1991;32:1741–1753.
39. Bjornheden T, Babyi A, Bondjers G, Wiklund O. Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. *Atherosclerosis*. 1996;123:43–56.
40. Nordestgaard BG, Zilversmit DB. Comparison of arterial intimal clearances of LDL from diabetic and nondiabetic cholesterol-fed rabbits: differences in intimal clearance explained by size differences. *Arteriosclerosis*. 1989;9:176–183.
41. Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis*. 1992;93:189–199.
42. St Pierre AC, Cantin B, Dagenais GR, Mauriege P, Bernard PM, Despres JP, Lamarche B. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec Cardiovascular Study. *Arterioscler Thromb Vasc Biol*. 2005;25:553–559.
43. Sacks FM, Campos H. Clinical review 163: cardiovascular endocrinology: low-density lipoprotein size and cardiovascular disease: a reappraisal. *J Clin Endocrinol Metab*. 2003;88:4525–4532.
44. Lada AT, Rudel LL. Associations of low density lipoprotein particle composition with atherogenicity. *Curr Opin Lipidol*. 2004;15:19–24.

CLINICAL PERSPECTIVE

The MetSyn is a clustering of cardiovascular risk factors and metabolic abnormalities. Persons with the MetSyn typically do not have elevated levels of LDL-C. However, it is likely that the atherogenic potential of LDL is defined not only by the cholesterol concentration of this class of lipoproteins but also by the size and number of LDL particles. NMR analysis has been developed to directly measure the size and concentration of plasma lipoproteins. In nearly 3000 participants of the Framingham Heart Study, we defined the relationship between LDL size and LDL particle concentrations, measured by NMR, and the number of components of the MetSyn. In both men and women, we observed that LDL particle numbers and especially small LDL particle concentrations increased almost linearly as the number of MetSyn components increased. The increase in LDL particles was not paralleled by an increase in LDL-C concentrations, which largely remained stable in a relatively low range. An increase in small LDL particle numbers was especially strongly correlated with higher triglycerides and lower levels of HDL-C. Although an increase in small LDL particle numbers defined the presence of the MetSyn with high specificity (≈85%), in this Framingham sample, individuals with the MetSyn and higher small LDL particle numbers (above the median) had CVD event rates similar to those with the MetSyn and lower small LDL particle numbers (below the median). This study demonstrates that with an increasing number of MetSyn components and despite little change in LDL-C concentrations, there is an increase in small LDL particle numbers, which are recognized to have a strong atherogenic potential.

Increased Small Low-Density Lipoprotein Particle Number: A Prominent Feature of the Metabolic Syndrome in the Framingham Heart Study

Sekar Kathiresan, James D. Otvos, Lisa M. Sullivan, Michelle J. Keyes, Ernst J. Schaefer, Peter W.F. Wilson, Ralph B. D'Agostino, Ramachandran S. Vasan and Sander J. Robins

Circulation. 2006;113:20-29; originally published online December 27, 2005;
doi: 10.1161/CIRCULATIONAHA.105.567107
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539