

## 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 ( $\geq 3$  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,<sup>1,2</sup> is known to be associated with an increased risk for cardiovascular disease (CVD).<sup>3,4</sup> It remains controversial whether the adverse risk associated with the MetSyn is greater than the sum of its parts.<sup>5-8</sup> 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.<sup>9</sup> ATP III defined the MetSyn by adverse levels of triglycerides, HDL cholesterol (HDL-C), blood pressure, glucose, and abdominal obesity.<sup>10</sup>

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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.<sup>11</sup> 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).<sup>9</sup>

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

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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.

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

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teins that transport cholesterol in the blood, including small LDL particles.<sup>12</sup> 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.<sup>13</sup>

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.<sup>14</sup> 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  $\geq 400$  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  $\geq 140$  mm Hg, a diastolic blood pressure  $\geq 90$  mm Hg, or use of antihypertensive therapy. Diabetes was defined as a fasting plasma glucose value  $\geq 126$  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.<sup>15</sup>

Participants were classified as having the MetSyn according to the 2004 revision of ATP III criteria.<sup>10</sup> 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  $\geq 150$  mg/dL; (3) HDL-C  $< 40$  mg/dL in men or  $< 50$  mg/dL in women; (4) systolic blood pressure  $\geq 130$  mm Hg, diastolic blood pressure  $\geq 85$  mm Hg, or use of antihypertensive therapy; and (5) fasting glucose  $\geq 100$  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.<sup>16</sup> 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  $-70^\circ$  by a commercially available proton NMR spectroscopic assay (LipoScience) as described previously.<sup>17,18</sup> 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)
<b>Clinical</b>		
Age, y	51 $\pm$ 10	51 $\pm$ 10
SBP, mm Hg	129 $\pm$ 17	124 $\pm$ 19
DBP, mm Hg	82 $\pm$ 10	77 $\pm$ 10
Fasting glucose, mg/dL	97 $\pm$ 26	92 $\pm$ 22
Waist circumference, cm	97 $\pm$ 11	81 $\pm$ 13
Diabetes, %*	7	4
Hypertension, %	38	29
<b>MetSyn criteria†</b>		
SBP $\geq 130$ mm Hg or DBP $\geq 80$ 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 $\geq 150$ mg/dL, %	29	16
Fasting glucose $\geq 100$ mg/dL, %†	19	11
ATP III-defined MetSyn, %	27	17
<b>Biochemical lipid measures</b>		
LDL-C, mg/dL	134 $\pm$ 33	127 $\pm$ 36
HDL-C, mg/dL	44 $\pm$ 12	56 $\pm$ 15
Triglycerides, mg/dL	126 $\pm$ 72	101 $\pm$ 57
ApoB, mg/dL	102 $\pm$ 23	94 $\pm$ 24
<b>NMR-derived lipoprotein measures</b>		
LDL particle size, nm	20.7 $\pm$ 0.6	21.1 $\pm$ 0.4
LDL particle No., nmol/L	1526 $\pm$ 409	1360 $\pm$ 425

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

\*Includes treated diabetics.

†Criteria as defined in Grundy et al<sup>10</sup>, 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\***

Variables	Men			Women		
	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 <sup>2</sup>	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),<sup>17</sup> 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.<sup>19</sup> 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.

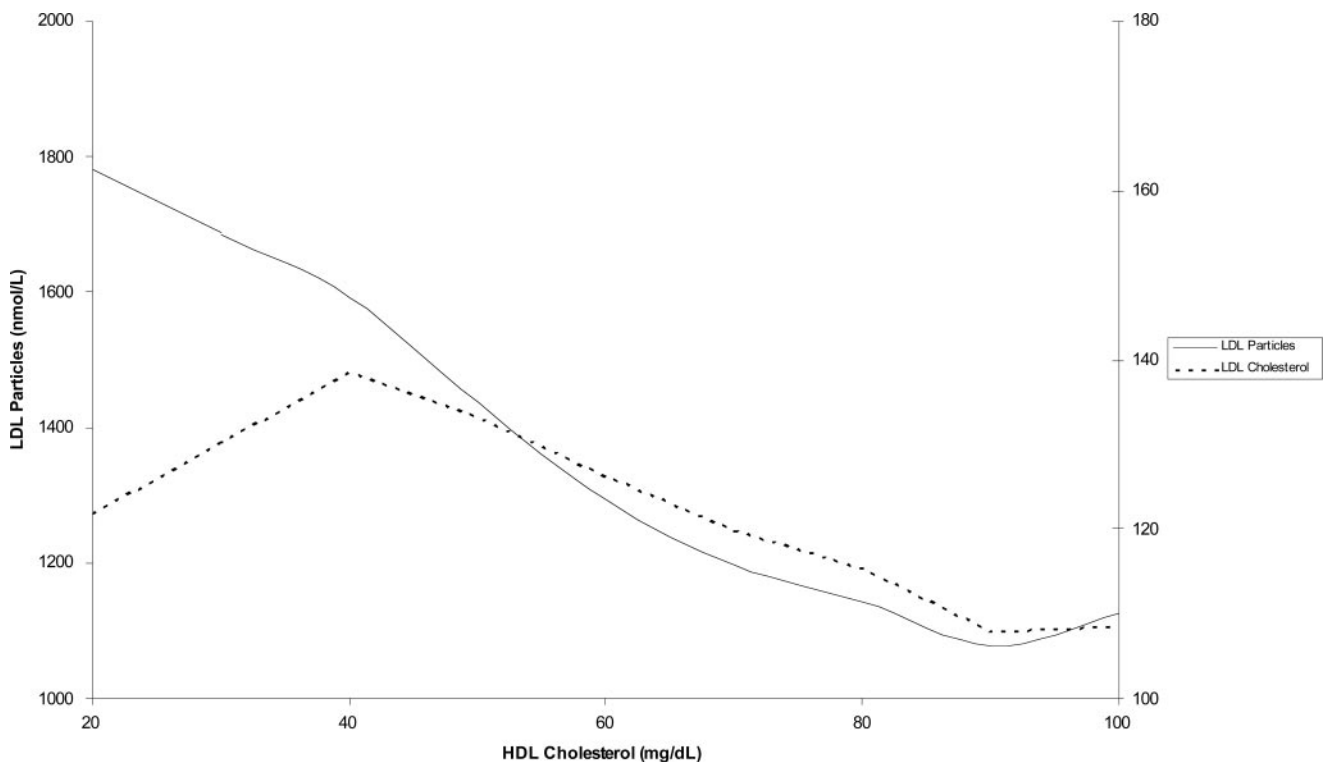
and the following set of covariates: for model 1, age, sex, and small LDL particle number and for model 2, age, sex, and apoB.

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.<sup>20</sup> We evaluated the sensitivity, specificity, positive predictive value, and negative predictive value for select LDL particle number cutpoints for identifying the MetSyn.

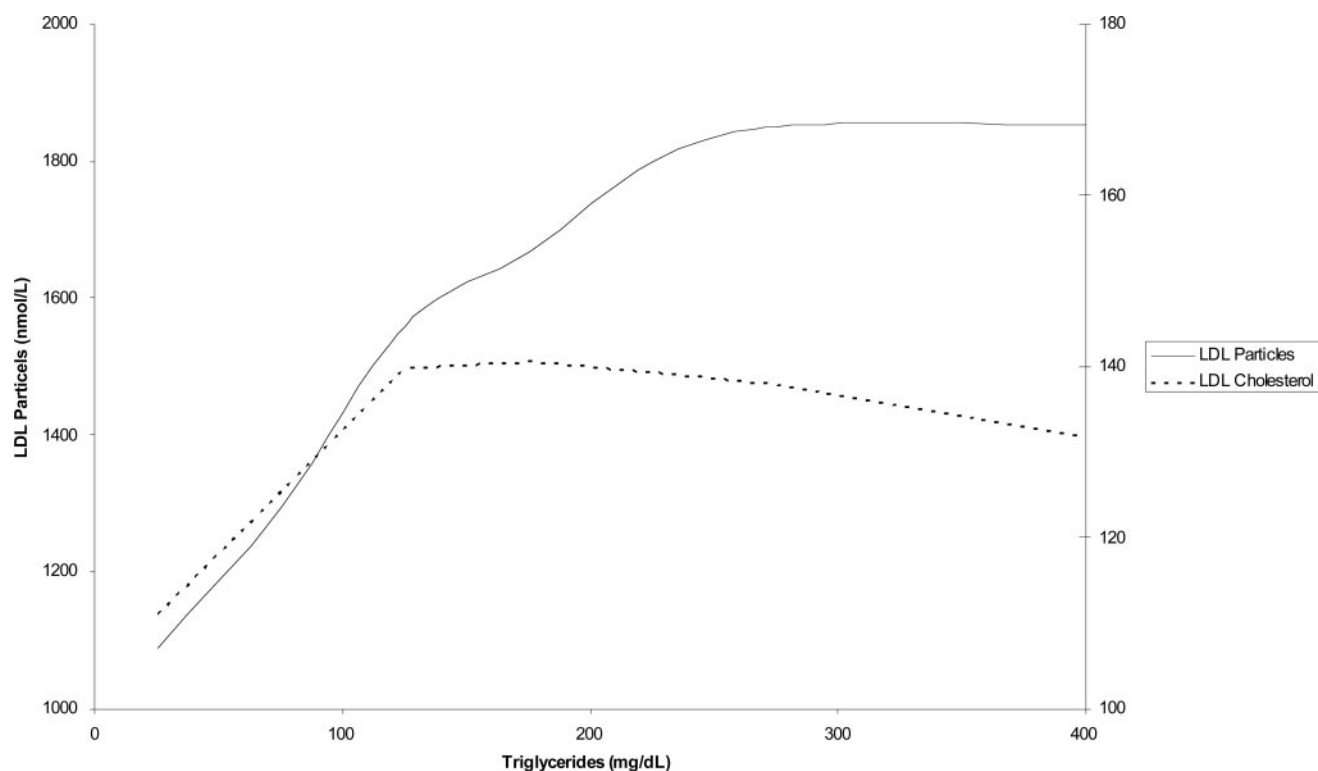
To assess whether CVD incidence among those with the MetSyn varied according to small LDL particle number or LDL-C, we calculated age-adjusted CVD event rates for 3 strata using Cox

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  $\approx 300$  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						P for Trend
	0	1	2	3	4	5	
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

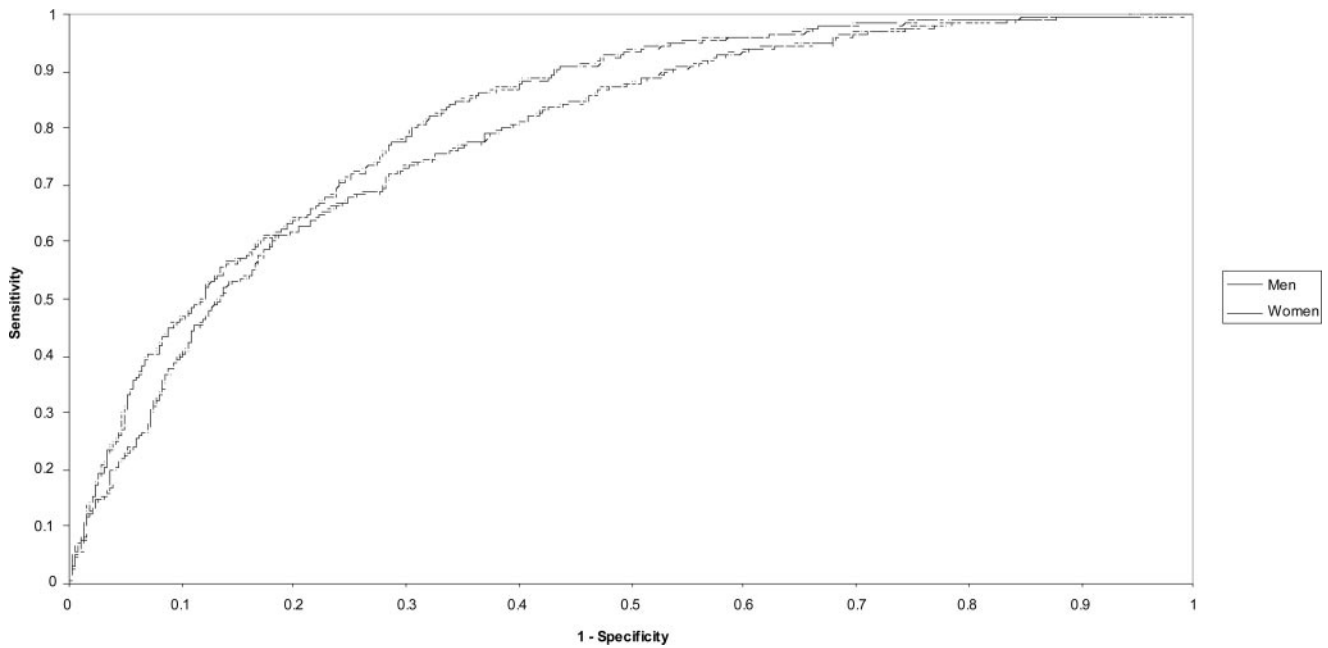
### Principal Findings

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.<sup>21</sup> Furthermore, with gel electrophoresis, small, dense LDL particles have also been associated with increased triglycerides, lower HDL-C, and increased apoB levels.<sup>11,22,23</sup> 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<sup>24</sup> and is a good surrogate measure for increased LDL particle numbers in people with diabetic dyslipidemia<sup>24</sup> as well as with the MetSyn and insulin resistance.<sup>25,26</sup>



**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<sup>27</sup> 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<sup>28</sup> 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
<b>Men*</b>				
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)
<b>Women†</b>				
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.<sup>29</sup> Several investigators have demonstrated that insulin resistance is associated with increased small LDL particle numbers. In the Insulin Resistance Atherosclerosis Study, Goff et al<sup>30</sup> 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,<sup>31</sup> 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.<sup>24,32</sup> 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.<sup>33</sup> 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.<sup>34</sup> 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.<sup>35</sup> The metabolic model described seems best to integrate the steady-state and dynamic lipoprotein studies available to date (reviewed in Berneis and Krauss<sup>36</sup> and Packard<sup>37</sup>).

**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,<sup>38</sup> entering the arterial wall more easily<sup>39,40</sup> and showing greater susceptibility to oxidation than large LDL.<sup>41</sup> 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;<sup>36,42</sup> however, this hypothesis has been challenged.<sup>43</sup>

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).<sup>43</sup> In this context, small LDL particle number may serve as marker of a metabolic condition and not necessarily directly promote atherosclerosis.<sup>44</sup>

### 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.

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### 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 ( $\approx 85\%$ ), 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

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