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Lipoprotein Subclass Abnormalities and Incident Hypertension in Initially Healthy Women

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Abstract

Background—Abnormalities in traditional lipids, particularly decreased high-density lipoprotein (HDL) cholesterol and increased triglycerides, precede the onset of hypertension. Whether lipoprotein particle size or subclass concentrations play a role in the development of hypertension is unknown.

Methods—17,527 initially healthy women without baseline hypertension were followed prospectively for 8 years. At baseline, traditional lipids and hypertension risk factors were obtained, and lipoprotein size and subclass concentrations were measured by nuclear magnetic resonance spectroscopy

Results—Baseline lipoprotein size and subclass concentrations were significantly associated with incident hypertension. While low density-lipoprotein (LDL) cholesterol was not associated with hypertension (odds ratio [OR] for quintile 5 vs 1: 1.08 [95% CI 0.96–1.20]), the concentration of LDL particles was associated with greater risk (OR 1.73 [1.54–1.95]), especially small LDL particles (OR 1.62 [1.45–1.83]). Increased HDL cholesterol was associated with lower risk of hypertension (OR for quintile 5 vs 1: 0.79 [0.70–0.89]). By contrast, increased concentration of HDL particles had greater risk (OR 1.48 [1.32–1.67]), especially small HDL particles (OR 1.36 [1.22–1.53]), while large HDL particles had lower risk (OR 0.80 [0.71–0.90]). Triglycerides and triglyceride-rich very-low-density lipoprotein (VLDL) particles were positively associated with hypertension, with large VLDL particles associated with greater risk (OR 1.68 [1.50–1.89]. Adding particle subclasses improved discrimination over a model with traditional lipids and risk factors (c-statistic 0.671 to 0.676, p-value <0.001).

Conclusion—In this study of initially healthy women, lipoprotein particle size and subclass concentrations were associated with incident hypertension and provided additive information to traditional lipids and risk factors.

Keywords

HDL particle size; hypertension; lipoproteins; LDL particle size; women

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Dr Otvos is employed by, is a stockholder of, and serves on the board of directors of LipoScience, Inc., a diagnostic laboratory company that performed the lipoprotein subclass analyses described in the manuscript. The other authors have no financial disclosures.

INTRODUCTION

Hypertension is a major risk factor for cardiovascular disease, affecting approximately 1 in 3 adults in the United States, with direct and indirect costs estimated at $43.5 billion in 2007.(1) Hypertension often clusters with dyslipidemia, especially among individuals with insulin resistance.(2) Previous studies found an increased risk of hypertension with lower concentration of high-density lipoprotein (HDL) cholesterol and higher concentrations of low-density lipoprotein (LDL) cholesterol and triglycerides.(3–6)

Lipoprotein abnormalities, particularly smaller LDL size or increased concentrations of triglyceride-rich particles, may contribute to high blood pressure by impairing endothelial function and promoting insulin resistance and vascular inflammation.(7–10) It has been recognized that a key feature of insulin resistance is the occurrence of a particular pattern of abnormalities in lipoprotein subclass distributions which is not detected by traditional lipid testing but which can be assessed using advanced lipoprotein testing such as nuclear magnetic resonance (NMR) spectroscopy of plasma.(11) Nuclear magnetic resonance (NMR) spectroscopy allows the simultaneous determination of both the concentration and size of lipoprotein particles. NMR measured lipoproteins have been shown to associate with the risk of cardiovascular disease(12–14), insulin resistance (8), and diabetes(15).

We hypothesized that lipoprotein particle size or subclass abnormalities that are associated with insulin resistance (i.e. smaller size of LDL and HDL particles, and increased concentration of triglyceride-rich particles) would predict incident hypertension. Therefore, in a large prospective cohort of initially healthy women, we evaluated the relationship between incident hypertension and baseline lipoprotein subclass size and concentration of LDL, HDL and very-low-density lipoprotein (VLDL) particles, and how they compared with traditional lipids or apolipoproteins. We further examined whether the association could be attenuated by biomarkers of inflammation/endothelial function, hyperglycemia, or other risk factors.

MATERIALS AND METHODS

Study Population

Study participants were from the Women's Health Study (WHS), a trial begun in 1992 to study the primary prevention of cardiovascular disease and cancer in initially healthy U.S. female health professionals aged 45 years or older randomized to take vitamin E and aspirin.(16, 17) All WHS participants provided written informed consent, and the study was approved by the institutional review board of the Brigham and Women's Hospital (Boston, Massachusetts).

A total of 28,345 (71%) WHS participants provided baseline blood samples. For this study, we excluded 7165 women who were hypertensive at baseline, defined as those who reported a systolic blood pressure greater than 140 mmHg, a diastolic blood pressure greater than 90 mmHg, any history of use of blood pressure medications, or any history of a physician diagnosis of hypertension. We further excluded women who were missing information on any lipid or lipoprotein measurements or other covariates, resulting in 17,527 women for this analysis.

Lipids and lipoprotein measures

EDTA blood samples were obtained at the time of enrollment into the WHS and stored in vapor phase liquid nitrogen (−170° C). The frozen plasma specimens were thawed and lipoprotein particle concentrations were measured by proton NMR spectroscopy (LipoScience, Inc., Raleigh, NC).(18, 19) NMR signal amplitudes of the spectroscopically

distinct lipid methyl group for each lipoprotein were used to calculate concentrations for the different lipoproteins.(18) The lipoprotein particles obtained included total HDL, further subdivided into large, medium and small particles; total LDL, further subdivided into large and small LDL particles and intermediate-density lipoprotein (IDL) particles; and total very low density lipoprotein (VLDL), further subdivided into large, medium and small particles. The IDL particles, sized between VLDL and LDL particles, were categorized with the LDL particles as they exhibit similar properties. Relative mass percentages were multiplied by the diameter of each subclass to obtain weighted average size for each lipoprotein particle. (18, 19) The NMR lipoprotein variables that we examined are those that are provided when ordering a commercially-available NMR lipoprotein profile.(20) Particle diameters and coefficients of variation (CVs) have been previously published for the NMR measures, with between-run CVs 7.1% or below for all particles except IDL (13%).(20)

All other plasma measurements were analyzed in a core laboratory facility certified by the National Heart, Lung and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization Program. Traditional lipid measures used in this study (total, HDL, and LDL cholesterol and tryglicerides) were all measured directly with a Hitachi 917 analyzer using reagents from Roche Diagnostics (Indianapolis, IN) with between-run CVs less than 3%. Apolipoproteins B100 (apoB) and A-1 (apoA-1) were measured using immunoturbidimetric assays (DiaSorin, Stillwater, Minn), with between-run CVs of 5% and 3%, respectively.

Hypertension

Baseline self-reported blood pressure (in mmHg categories of <110, 110–119, 120–129, 130–139, 140–149, 150–159, 160–169, 170–179, and ≥180 for systolic blood pressure and &x0003C;65, 65–74, 75–84, 85–89, 90–94, 95–104, and ≥105 for diastolic blood pressure), history of treatment for high blood pressure, and physician diagnosis of hypertension were assessed by questionnaire. Incident hypertension was ascertained by annual questionnaire using methods previously described in detail.(3) Briefly, participants were classified as hypertensive after reporting either a new physician diagnosis at year 1, 3, or annually thereafter; a new hypertensive treatment at year 1, 3, or 4; a systolic blood pressure of 140 mmHg or greater at year 1 or 4; or a diastolic blood pressure of 90 mmHg or greater at year 1 or 4. The reproducibility of self-reported hypertension status in these female health professionals was assessed in a sub-sample of participants using medical records, with high rates of agreement (96% confirmation rate for reports of hypertension and 90% confirmation rate for reports of no hypertension).(21)

Covariates

Baseline age, race, diabetes, alcohol use, exercise frequency, treatment for high cholesterol, postmenopausal hormone use, diet, education, smoking, and menopausal status were collected from self-reported questionnaires. Body mass index (BMI) was calculated from self-reported height and weight at baseline. Other markers relating to inflammation and endothelial function including C-reactive protein, fibrinogen, homocysteine, and soluble intercellular adhesion molecule-1, as well as hemoglobin A1c levels, were also measured as previously described.(22)

Statistical Methods

Logistic models with an outcome of incident hypertension at 8 years were chosen as the primary modeling strategy. Lipid measurements were divided into quintiles and were analyzed both categorically and for linear trend across quintiles using quintile number. The primary adjustment for confounding included non-lipid risk factors (baseline values of age, smoking, fasting status, use of cholesterol lowering medication, trial treatment assignment,

hormone use, menopausal status, race, exercise, alcohol use, BMI, diabetes, education, vegetable, fruit, sodium, and total grain intake). The increase in the likelihood ratio obtained by adding the lipid measurement to the non-lipid risk factors was also derived.

To assess the independent impact of each of the NMR lipoprotein sizes, a fully adjusted model including all 9 lipoprotein subclasses was examined. A fully adjusted model was also examined using mean size and particle concentration for each lipoprotein plus non-lipid risk factors. C-statistics were used to compare the addition of NMR measures to models with non-lipid risk factors and traditional lipids.(23) A similar analysis was also performed with standard lipids and apolipoprotein measures plus non lipid risk factors.

In order to assess potential mediators, we examined models with additional adjustment for inflammatory/endothelial function markers (C-reactive protein, fibrinogen, homocysteine, and soluble intercellular adhesion molecule-1), hemoglobin A1c levels, and baseline blood pressure.

All analyses were also redone in designated subgroups and tested for interaction: 1) BMI divided into obese (BMI greater than or equal to 30), overweight (BMI of 25 to 30) and normal (BMI of less than 25)(24); 2) blood pressure less than 120 / 70 mmHg; 3) metabolic syndrome categories previously used in the Women's Health Study(25) with and without the blood pressure criterion of ≥ 130/85 mmHg; 4) non-users of lipid lowering medication.

All analyses were done using R version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

During 8 years of follow-up, 4714 cases (27%) of incident hypertension occurred. As shown in Table 1, women who developed hypertension were older at baseline with higher baseline blood pressures, along with a higher prevalence of hormone use, diabetes, and postmenopausal status. They also exercised less, had a higher BMI, and were more likely to be Black or Hispanic.

Median baseline lipid and lipoprotein measures significantly differed in women who went on to develop hypertension. HDL cholesterol, apoA-1, the concentration of large LDL and HDL particles, and the average LDL and HDL particle size were lower in women who developed hypertension. All other lipid and lipoprotein measures were higher in women who developed hypertension. Correlations between the NMR and traditional lipid measures were similar to previously published values for the WHS (12), with low correlations between the average sizes and total particle numbers and low to moderate correlations among the 9 particle subclasses.

LDL Measures

In unadjusted analysis by quintile of each measure, all LDL measures were associated with hypertension, as shown in Table 2. After adjustment for non-lipid risk factors (Model 1: age, smoking, fasting status, use of cholesterol lowering medication, trial treatment assignment, hormone use, menopausal status, race, exercise, alcohol use, BMI, diabetes, education, vegetable, fruit, sodium, and total grain intake), LDL cholesterol was no longer associated. By contrast, apoB and all of the LDL NMR measures remained significantly associated with incident hypertension. Large LDL particle concentration and average LDL particle size were inversely associated with risk of hypertension, while small LDL, IDL, and hence total concentration of LDL particles were associated with increased risk. Of note, the largest odds

ratios and likelihood ratios for comparing quintile 5 to quintile 1 were for total LDL particle concentration (OR 1.73, LR χ^2 105.61) and small LDL particles (OR 1.62, LR χ^2 96.32).

The results were essentially unchanged after additional adjustments for baseline inflammatory/endothelial biomarkers (C-reactive protein, fibrinogen, homocysteine, and soluble intercellular adhesion molecule 1) and hemoglobin A1c (Model 2 results, Table 2).

HDL Measures

All HDL measures were associated with incident hypertension in unadjusted analyses (Table 3). After adjustment for non lipid risk factors (Model 1), apoA-1 was no longer independently associated, while standard HDL cholesterol and all HDL NMR measures remained significantly associated. The total concentration of HDL particles, specifically the medium and small HDL particles which make up most of the total concentration of HDL, were associated with increased risk, while large HDL particles were inversely associated with risk of hypertension. Accordingly, larger average HDL particle size was also associated with decreased incidence of hypertension. The largest odds ratios and likelihood ratios for comparison of quintile 5 to quintile 1 were for HDL particle size (OR 0.66, LR χ^2 66.16) and total HDL particle concentration (OR 1.48, LR χ^2 54.81). Further adjustment for inflammatory/endothelial biomarkers and hemoglobin A1c did not alter the magnitude of association (Model 2 results, Table 3).

VLDL and Triglyceride Measures

The VLDL and triglyceride measures (Table 4) were all associated with hypertension prior to adjustment and all except small VLDL particle concentration remained associated after adjustment for non lipid risk factors (Model 1). In contrast with the association of smaller size of LDL and HDL with hypertension, larger VLDL size and large VLDL particle concentration were associated with increased risk of hypertension. The largest odds ratios and likelihood ratios for comparison of quintile 5 to quintile 1 were for triglycerides (OR 1.65, LR χ^2 91.63) and large VLDL particles (OR 1.68, LR χ^2 90.73). Similar results were obtained after additionally adjusting for the inflammatory/endothelial biomarkers and hemoglobin A1c (Model 2 results, Table 4).

Mutually-adjusted Effects of Lipoprotein Particle Subclasses

In a model including quintiles of NMR particle concentrations for the 9 non-overlapping particle subclasses and non-lipid risk factors, the medium and small HDL particles, the IDL and small LDL particles, and the medium and large VLDL particles remained independently associated with hypertension risk (Figure 1). Consistent with the previous results, these particle subclasses, with the exception of medium VLDL particles, were associated with an increased risk of hypertension.

Mutually-adjusted Effects of Lipoprotein Particle Concentration versus Size

When the total particle concentration and average particle size for each lipoprotein type were combined into one model with non-lipid risk factors, the total concentration of LDL and HDL particles and average VLDL particle size were each independently associated with increased risk of hypertension. (Figure 2) These results are consistent with the finding that of the VLDL particles, the large particles were associated with the greatest increase in risk.

Adding lipoproteins to traditional lipids

Addition of the nine particle subclasses improved discrimination over a base model with non-lipid factors and traditional lipids, increasing the c-statistic from 0.671 to 0.676 ($p < 0.001$). In a separate analysis, adding the total particle concentration and average particle

size for each lipoprotein type over a base model with non-lipid risk factors and traditional lipids also improved the c-statistic to 0.677 (p = 0.001 for comparison to the base model). Addition of apoA-1 and apoB to the base model did not statistically significantly improve the c-statistic (0.673, p=0.7), although both apolipoproteins remained independently associated with incident hypertension.

Subgroup Analyses

Results were similar to the main study results in each of the pre-specified subgroup analysis (BMI categories, low baseline blood pressure, presence of metabolic syndrome, and non-users of cholesterol lowering medications). Additionally there was no evidence of interactions with any subgroup. The BMI subgroup results are shown in Supplemental Table 1. Our results also remained similar after additional adjustments for baseline blood pressure.

DISCUSSION

This study involving 17,527 initially healthy women followed prospectively for 8 years is the first to document that the pattern of lipoprotein subclass abnormalities that predicted incident hypertension are the same pattern that has been previously found to be characteristic of insulin resistance and diabetes (i.e. higher concentrations of small LDL, small HDL, and large VLDL particles).(11, 15) Additionally, we found that the total concentration of LDL, HDL and VLDL particles was significantly associated with increased risk and provided additional information to the traditional lipid panel and other risk factors for hypertension.

Postulated mechanisms for the relationship between lipoprotein patterns and incident hypertension include the common pathways of insulin resistance, inflammation and endothelial dysfunction by increasing the endothelial oxidative burden.(7–10, 26) We explored additional adjustment for inflammation/endothelial markers including Creactive protein, fibrinogen, homocysteine, and soluble intercellular adhesion molecule-1, since some of these biomarkers have been related to incident hypertension.(27) Further adjustment for inflammation/endothelial markers did not affect the associations of lipoprotein size and concentrations with hypertension. Similarly, adjustment for hemoglobin A1c did not alter the results. These findings suggest that the mechanisms of increased hypertension risk associated with lipoprotein abnormalities are unlikely to be mediated by these biomarkers of inflammation/endothelial function or dysglycemia.

Traditional lipid measures have been shown to be associated with increased risk of hypertension in this and other cohorts.(3–6) In particular, lower HDL cholesterol and higher triglycerides (and in some studies, LDL cholesterol) have been associated with increased risk. Triglycerides and apoB were also shown to be positively associated with an increased risk of hypertension in middle-aged Finnish men.(6) Our study confirms these findings for HDL cholesterol, apoB, and triglycerides in this middle-aged and older cohort of women, although we did not find LDL cholesterol to be independently related to incident hypertension.

Previous studies in both this cohort (12, 15) and others (13, 14) have linked NMR lipoprotein measures to cardiovascular disease and diabetes. Consistent with our results, increased concentration of LDL particles, specifically small particles, has been shown to increase cardiovascular risk as well as risk of incident type 2 diabetes, as has increased concentration of small HDL particles. These patterns are consistent with a shared insulin resistance pathway. We did not have a specific measure for insulin resistance in our study, although adjustment for BMI, triglycerides, hemoglobin A_{1c} and inflammatory biomarkers did not substantially change the results.

The study benefits from a large sample size with well-characterized subjects and a long follow-up (8 years). However, since our study is limited to women, the generalizability of our results to men remains unclear, though traditional lipids have been found to associate with hypertension in both groups. Additionally, our measure of hypertension was self-reported. While this may introduce variability into the outcome measure, we believe this variability is unlikely to be related to lipoprotein measures and is therefore unlikely to affect the direction of our results. The large sample size of the study is also helpful in providing sufficient power to observe associations despite measurement variability. Self-reported hypertension has been shown to be a valid and reliable measure in this(28), as well as other cohorts of health professionals.(29)

In summary, we found that among initially healthy women, lipoprotein particle size and subclass concentrations were associated with incident hypertension and provided additive information to traditional lipids and risk factors. Greater risk of hypertension was associated with higher total concentrations of LDL and HDL particles, especially small particles, and higher total concentration of VLDL, especially large particles. Further, our findings suggest that the concentrations and size of lipoprotein particles affect the risk of incident hypertension years before the clinical onset of hypertension, even in women with initially normal blood pressure. Further research is necessary to determine whether treatment based on lipoprotein profiles would reduce incident hypertension. However, the possibility of identification of a subgroup at increased risk for incident hypertension using lipoprotein measures assessed years before the onset of clinical hypertension may be of use for patients and clinicians. This additional information beyond traditional lipid measures may be useful both in understanding the etiology and in complementing the use of traditional risk factors for predicting the risk of incident hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

HDL	high-density lipoprotein
LDL	low-density lipoprotein
OR	odds ratio
VLDL	very-low-density lipoprotein
NMR	nuclear magnetic resonance
WHS	Women’s Health Study
IDL	intermediate-density lipoprotein
CVs	coefficients of variation
apoB	apolipoprotein B100
apoA-1	apolipoprotein A-1
BMI	body mass index
LR	likelihood ratio

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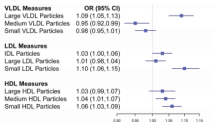

Figure 1.

Mutually Adjusted Effects Per Quintile for Individual NMR Lipoprotein Subclasses on Incident Hypertension. All odds ratios are from a single model including NMR subclasses and non-lipid risk factors. Statistically significant p-values were noted for IDL (0.03), small LDL (<0.001), medium HDL (0.005), small HDL (<0.001), large VLDL (<0.001) and medium VLDL (0.005).

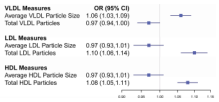

Figure 2.

Mutually Adjusted Effects Per Quintile for NMR Lipoprotein Size and Total Concentration on Incident Hypertension. All odds ratios are from a single model including NMR measures and non-lipid risk factors. Statistically significant p-values were noted for total LDL particles (<0.001), total HDL particles (<0.001), average VLDL particle size (<0.001) and total VLDL particles (0.033).

Table 1

Baseline Characteristics of 17,572 Women Initially Free of Hypertension

	Incident hypertension at 8 years (N = 4714)	No hypertension at 8 years (N = 12858)	P-value*
Age, years	53.6 (49.4,59.6)	51.6 (48.2,56.6)	< 0.001
Systolic blood pressure, mm Hg	125 (115,135)	115 (105,125)	< 0.001
Diastolic blood pressure, mm Hg	80 (70,80)	70 (70,80)	< 0.001
Current hormone use, %	46.0	42.8	< 0.001
Diabetes, %	2.7	0.9	< 0.001
Postmenopausal, %	56.5	48.4	< 0.001
Black or Hispanic, %	2.9	1.9	< 0.001
Current cigarette smoker, %	11.5	11.4	0.88
Alcohol use, %			< 0.001
Rarely/never	45.3	40.5	
1–3 drinks/month	13.2	13.5	
1–6 drinks/week	31.2	35.4	
1+ drinks/day	10.2	10.6	
Exercise frequency, %			< 0.001
Rarely/never	39.1	33.4	
<1 time/week	19.7	19.9	
1–3 times/week	31.0	33.9	
4+ times/week	10.2	12.8	
Body mass index, kg/m^2	25.7 (23.1,29.2)	23.7 (21.8,26.6)	< 0.001
Current cholesterol treatment, %	2.9	1.9	< 0.001
Total cholesterol, mg/dL	210 (186,238)	204 (181,231)	< 0.001
LDL Measures			
LDL cholesterol, mg/dL	122.6 (101.6,146.1)	118.2 (98.2,140.9)	< 0.001
Apolipoprotein B, mg/dL	105.1 (86.9,124.1)	95.3 (79.9,115.1)	< 0.001
NMR LDL particle concentration, nmol/L			
Total	1350 (1091,1679)	1191 (970,1476)	< 0.001
IDL	38 (14,74)	26 (8,59)	< 0.001
Large	535 (388,686)	556 (426,692)	< 0.001
Small	730 (450,1108)	566 (340,867)	< 0.001
Average NMR LDL particle size, nm	21.3 (20.7,21.8)	21.5 (21,22)	< 0.001
HDL Measures			
HDL cholesterol, mg/dL	50.7 (42.3,61.1)	54.2 (45.3,64.6)	< 0.001
Apolipoprotein A-I, mg/dL	149.2 (132.2,167.9)	150.3 (134,168.5)	0.007
NMR HDL particle concentration, μmol/L			
Total	35.5 (31.5,40)	34.6 (30.8,38.9)	< 0.001
Large	7.1 (4.6,10)	8.2 (5.6,10.8)	< 0.001
Medium	3.1 (0.9,6.5)	2.6 (0.7,5.8)	< 0.001
Small	24.1 (20.5,27.7)	22.9 (19.3,26.5)	< 0.001

	Incident hypertension at 8 years (N = 4714)	No hypertension at 8 years (N = 12858)	P-value*
Average NMR HDL particle size, nm	8.9 (8.6,9.3)	9.1 (8.8,9.4)	< 0.001
VLDL Measures			
Triglycerides, mg/dL	129 (90,187)	106 (75,153)	< 0.001
NMR VLDL particle concentration, nmol/L			
Total	70.9 (51.3,93.2)	65.3 (46.7,86.7)	< 0.001
Large	1.9 (0.5,4.2)	1.0 (0.2,2.9)	< 0.001
Medium	22.0 (12.0,33.4)	19.6 (10.3,30.6)	< 0.001
Small	45.9 (32.9,58.6)	43.1 (31.1,56.4)	< 0.001
Average NMR VLDL particle size, nm	47.6 (42.9,53)	45.5 (41.6,50.7)	< 0.001

Values shown are median (25 percentile, 75 percentile) unless otherwise indicated

*Kruskal-Wallis for continuous variables, Chi-squared for categorical

Table 2

Association of LDL Measures with Incident Hypertension

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	LR Chi2	P for trend
LDL Cholesterol mg/dL	**23.7 – 94.6**	**94.7 – 111.6**	**111.7 – 127.7**	**127.8 – 148.6**	**148.7 – 335.4**		
Unadjusted	1	1.06 (0.95,1.18)	1.21 (1.08,1.34)	1.28 (1.15,1.42)	1.45 (1.30,1.61)	59.37	<0.001
Model 1	1	0.97 (0.87,1.09)	1.04 (0.93,1.16)	1.03 (0.92,1.15)	1.08 (0.96,1.20)	3.58	0.11
Model 2	1	0.97 (0.87,1.09)	1.04 (0.93,1.16)	1.03 (0.92,1.15)	1.07 (0.96,1.2)	3.32	0.13
Apolipoprotein B, mg/dL	**21.8 – 78**	**78.0 – 91.2**	**91.3 – 106.2**	**106.3 – 122.8**	**122.9 – 257.4**		
Unadjusted	1	1.12 (1.00,1.26)	1.45 (1.30,1.63)	1.69 (1.51,1.88)	2.23 (2.00,2.48)	282.03	<0.001
Model 1	1	0.99 (0.88,1.11)	1.14 (1.01,1.28)	1.23 (1.10,1.38)	1.44 (1.28,1.61)	57.68	<0.001
Model 2	1	0.99 (0.88,1.11)	1.13 (1.00,1.26)	1.21 (1.08,1.36)	1.39 (1.24,1.56)	46.52	<0.001
NMR particle concentration, nmol/L							
Total LDL Particles	**303 – 947**	**948 –1135**	**1136 – 1335**	**1336 – 1624**	**1625 – 4405**		
Unadjusted	1	1.29 (1.15,1.45)	1.59 (1.42,1.78)	2.09 (1.87,2.34)	2.82 (2.53,3.15)	444.67	<0.001
Model 1	1	1.13 (1.00,1.27)	1.23 (1.09,1.39)	1.46 (1.30,1.64)	1.73 (1.54,1.95)	105.61	<0.001
Model 2	1	1.11 (0.99,1.26)	1.20 (1.07,1.36)	1.41 (1.26,1.59)	1.63 (1.45,1.84)	82.09	<0.001
IDL Particles	**0 – 5**	**6 – 20**	**21 – 40**	**41 – 73**	**74 – 339**		
Unadjusted	1	1.12 (1.00,1.25)	1.36 (1.22,1.52)	1.67 (1.50,1.86)	1.95 (1.75,2.17)	209.03	<0.001
Model 1	1	1.02 (0.91,1.15)	1.16 (1.03,1.30)	1.3 (1.16,1.46)	1.35 (1.20,1.51)	44.52	<0.001
Model 2	1	1.01 (0.9,1.14)	1.14 (1.01,1.28)	1.25 (1.12,1.41)	1.29 (1.15,1.44)	32.10	<0.001
Large LDL Particles	**0 – 384**	**385 – 500**	**501 – 603**	**604 – 731**	**732 – 2917**		
Unadjusted	1	0.72 (0.65,0.80)	0.70 (0.64,0.78)	0.64 (0.57,0.71)	0.74 (0.66,0.82)	82.85	<0.001
Model 1	1	0.82 (0.73,0.91)	0.83 (0.74,0.92)	0.75 (0.67,0.83)	0.82 (0.74,0.92)	29.49	<0.001
Model 2	1	0.84 (0.75,0.93)	0.85 (0.77,0.95)	0.77 (0.69,0.86)	0.85 (0.76,0.95)	22.94	0.001
Small LDL Particles	**0 – 313**	**314 – 507**	**508 – 712**	**713 – 1040**	**1041 – 3457**		
Unadjusted	1	1.13 (1.01,1.27)	1.37 (1.22,1.53)	1.91 (1.71,2.13)	2.51 (2.25,2.79)	398.83	<0.001
Model 1	1	1.06 (0.94,1.20)	1.17 (1.04,1.32)	1.44 (1.28,1.61)	1.62 (1.45,1.83)	96.32	<0.001
Model 2	1	1.05 (0.93,1.19)	1.15 (1.02,1.29)	1.39 (1.24,1.56)	1.53 (1.36,1.73)	72.90	<0.001
Average NMR LDL Size, nm	**19.0 – 20.8**	**20.9 – 21.2**	**21.3 – 21.6**	**21.7 – 22**	**22.1 – 23.0**		
Unadjusted	1	0.72 (0.65,0.80)	0.61 (0.55,0.67)	0.51 (0.46,0.57)	0.44 (0.39,0.48)	301.70	<0.001
Model 1	1	0.86 (0.77,0.96)	0.78 (0.70,0.86)	0.71 (0.64,0.79)	0.64 (0.57,0.72)	70.82	<0.001

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	LR Chi2	P for trend
Model 2	1	0.88 (0.79,0.98)	0.81 (0.73,0.89)	0.75 (0.67,0.83)	0.68 (0.6,0.76)	51.50	<0.001

Ranges (minimum – maximum) and odds ratios with 95% confidence intervals are given for each quintile.
Model 1 includes age, smoking, fasting status, use of cholesterol lowering medication, trial treatment assignment, hormone use, menopausal status, race, exercise, alcohol use, body mass index, diabetes, education, vegetable, fruit, sodium, and total grain intake.
Model 2 adds C-reactive protein, homocysteine, fibrinogen, soluble inter-cellular adhesion molecule 1, and hemoglobin A1C to Model 1

Table 3

Association of HDL Measures with Incident Hypertension

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	LR Chi2	P for trend
HDL Cholesterol, mg/dL	**15.9 – 42.4**	**42.5 – 49.7**	**49.9 – 56.9**	**57.0 – 66.5**	**66.6 –173.0**		
Unadjusted	1	0.79 (0.72,0.88)	0.66 (0.59,0.73)	0.58 (0.52,0.64)	0.54 (0.49,0.60)	174.83	<0.001
Model 1	1	0.94 (0.84,1.04)	0.85 (0.76,0.94)	0.78 (0.70,0.88)	0.79 (0.70,0.89)	25.25	<0.001
Model 2	1	0.96 (0.86,1.06)	0.87 (0.78,0.97)	0.8 (0.72,0.9)	0.81 (0.71,0.91)	20.73	<0.001
Apolipoprotein A-I, mg/dL	**49.8 –129**	**129.6 –143**	**143.4 –156**	**156.6 –172.9**	**173 – 249**		
Unadjusted	1	0.88 (0.80,0.98)	0.84 (0.76,0.94)	0.88 (0.80,0.98)	0.85 (0.77,0.95)	12.70	0.008
Model 1	1	0.96 (0.86,1.07)	0.98 (0.87,1.09)	1.07 (0.96,1.20)	1.03 (0.91,1.17)	4.63	0.23
Model 2	1	0.97 (0.87,1.09)	0.99 (0.88,1.11)	1.07 (0.96,1.21)	1.01 (0.89,1.14)	3.46	0.41
NMR particle concentration, µmol/L							
Total HDL Particles	**12.1 – 30.2**	**30.3 – 33.3**	**33.4 – 36.4**	**36.5 – 40.3**	**40.4 – 67.9**		
Unadjusted	1	1.02 (0.92,1.14)	1.13 (1.01,1.26)	1.24 (1.12,1.38)	1.47 (1.32,1.63)	69.19	<0.001
Model 1	1	1.02 (0.91,1.15)	1.12 (1.00,1.25)	1.23 (1.09,1.37)	1.48 (1.32,1.67)	54.81	<0.001
Model 2	1	1.02 (0.91,1.15)	1.12 (1.00,1.25)	1.21 (1.08,1.36)	1.43 (1.26,1.61)	41.88	<0.001
Large HDL Particles	**0 – 4.7**	**4.8 – 6.9**	**7.0 – 8.9**	**9.0 –11.3**	**11.4 –25.3**		
Unadjusted	1	0.74 (0.67,0.82)	0.62 (0.56,0.69)	0.51 (0.46,0.57)	0.56 (0.50,0.62)	200.89	<0.001
Model 1	1	0.88 (0.79,0.98)	0.83 (0.75,0.93)	0.73 (0.65,0.82)	0.80 (0.71,0.9)	30.21	<0.001
Model 2	1	0.90 (0.81,1.00)	0.86 (0.77,0.96)	0.76 (0.67,0.85)	0.81 (0.72,0.91)	24.29	<0.001
Medium HDL Particles	**0 – 0.5**	**0.6 – 1.8**	**1.9 – 3.8**	**3.9 – 7.0**	**7.1 – 30.4**		
Unadjusted	1	1.12 (1.01,1.25)	1.15 (1.03,1.28)	1.27 (1.14,1.41)	1.43 (1.29,1.59)	52.12	<0.001
Model 1	1	1.06 (0.95,1.18)	1.06 (0.95,1.18)	1.17 (1.05,1.30)	1.31 (1.17,1.46)	28.06	<0.001
Model 2	1	1.05 (0.94,1.18)	1.04 (0.93,1.17)	1.14 (1.02,1.27)	1.26 (1.12,1.41)	18.878	<0.001
Small HDL Particles	**0 – 18.7**	**18.8 – 21.9**	**22.0 – 24.5**	**24.6 – 27.7**	**27.8 – 49.9**		
Unadjusted	1	1.07 (0.96,1.19)	1.27 (1.14,1.42)	1.46 (1.31,1.63)	1.77 (1.59,1.97)	151.12	<0.001
Model 1	1	0.98 (0.87,1.10)	1.11 (0.99,1.25)	1.17 (1.04,1.30)	1.36 (1.22,1.53)	45.35	<0.001
Model 2	1	0.99 (0.88,1.11)	1.12 (1.00,1.25)	1.16 (1.04,1.30)	1.34 (1.20,1.50)	38.18	<0.001
Average NMR HDL Size, nm	**8.0 – 8.6**	**8.7 – 8.9**	**9.0 – 9.2**	**9.3 – 9.5**	**9.6 – 10.8**		
Unadjusted	1	0.73 (0.66,0.80)	0.57 (0.51,0.62)	0.49 (0.44,0.55)	0.40 (0.35,0.45)	338.06	<0.001
Model 1	1	0.85 (0.77,0.94)	0.75 (0.68,0.84)	0.72 (0.64,0.80)	0.62 (0.55,0.7)	66.16	<0.001

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	LR Chi2	P for trend
Model 2	1	0.86 (0.78,0.95)	0.77 (0.70,0.86)	0.74 (0.66,0.83)	0.65 (0.57,0.73)	52.73	<0.001

Ranges (minimum – maximum) and odds ratios with 95% confidence intervals are given for each quintile.
Model 1 includes age, smoking, fasting status, use of cholesterol lowering medication, trial treatment assignment, hormone use, menopausal status, race, exercise, alcohol use, body mass index, diabetes, education, vegetable, fruit, sodium, and total grain intake.
Model 2 adds C-reactive protein, homocysteine, fibrinogen, soluble inter-cellular adhesion molecule 1, and hemoglobin A1C to Model 1

Table 4

Association of VLDL and Triglyceride Measures with Incident Hypertension

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	LR Chi2	P for trend
Triglycerides, mg/dL	**16 – 73**	**74 – 97**	**98 – 129**	**130 – 178**	**179 – 954**		
Unadjusted	1	1.22 (1.09,1.38)	1.60 (1.43,1.79)	1.93 (1.73,2.16)	2.63 (2.36,2.93)	395.09	<0.001
Model 1	1	1.06 (0.94,1.20)	1.27 (1.13,1.43)	1.37 (1.22,1.54)	1.65 (1.47,1.86)	91.63	<0.001
Model 2	1	1.04 (0.92,1.17)	1.23 (1.09,1.38)	1.30 (1.15,1.47)	1.53 (1.35,1.73)	61.79	<0.001
NMR particle concentration, nmol/L							
Total VLDL Particles	**0.1 – 43.5**	**43.6 – 59.3**	**59.4 – 74.4**	**74.5 – 94.6**	**94.7 – 258.6**		
Unadjusted	1	1.06 (0.94,1.18)	1.26 (1.13,1.40)	1.39 (1.25,1.54)	1.57 (1.41,1.74)	96.21	<0.001
Model 1	1	0.96 (0.86,1.08)	1.06 (0.95,1.19)	1.09 (0.97,1.22)	1.16 (1.04,1.30)	13.54	<0.001
Model 2	1	0.96 (0.85,1.07)	1.05 (0.94,1.18)	1.07 (0.96,1.20)	1.14 (1.01,1.27)	10.57	0.004
Large VLDL Particles	**0 – 0.2**	**0.3 – 0.7**	**0.8 – 1.9**	**2.0 – 4.0**	**4.1 – 35.8**		
Unadjusted	1	1.25 (1.12,1.41)	1.55 (1.38,1.73)	2.01 (1.80,2.23)	2.57 (2.32,2.86)	388.79	<0.001
Model 1	1	1.16 (1.03,1.31)	1.28 (1.14,1.43)	1.44 (1.28,1.61)	1.68 (1.50,1.89)	90.73	<0.001
Model 2	1	1.16 (1.03,1.30)	1.25 (1.11,1.40)	1.38 (1.23,1.55)	1.59 (1.41,1.79)	65.95	<0.001
Medium VLDL Particles	**0 – 8.8**	**8.9 – 16.4**	**16.5 – 24.1**	**24.2 – 34.4**	**34.5 – 138.1**		
Unadjusted	1	1.10 (0.99,1.23)	1.28 (1.15,1.42)	1.29 (1.15,1.43)	1.53 (1.37,1.70)	72.79	<0.001
Model 1	1	1.00 (0.90,1.12)	1.10 (0.98,1.23)	1.08 (0.96,1.21)	1.19 (1.06,1.33)	13.03	0.001
Model 2	1	0.99 (0.88,1.11)	1.08 (0.96,1.21)	1.05 (0.94,1.18)	1.15 (1.03,1.29)	9.30	0.007
Small VLDL Particles	**0 – 28.7**	**28.8 – 39.2**	**39.3 – 48.7**	**48.8 – 60.6**	**60.7 – 157.8**		
Unadjusted	1	1.04 (0.93,1.16)	1.10 (0.99,1.22)	1.33 (1.20,1.48)	1.33 (1.20,1.48)	51.28	<0.001
Model 1	1	0.95 (0.85,1.07)	0.95 (0.85,1.06)	1.10 (0.98,1.23)	1.04 (0.93,1.17)	9.53	0.07
Model 2	1	0.95 (0.85,1.06)	0.95 (0.85,1.06)	1.09 (0.98,1.22)	1.04 (0.93,1.16)	9.28	0.09
Average NMR VLDL Size, nm	**31.8 – 41.0**	**41.1 – 44.4**	**44.5 – 48.0**	**48.1 – 52.9**	**53.0 – 131.2**		
Unadjusted	1	0.99 (0.88,1.11)	1.26 (1.13,1.40)	1.58 (1.42,1.76)	1.84 (1.66,2.05)	209.57	<0.001
Model 1	1	0.97 (0.86,1.09)	1.12 (1.00,1.25)	1.30 (1.16,1.45)	1.42 (1.27,1.59)	63.54	<0.001
Model 2	1	0.97 (0.86,1.09)	1.11 (0.99,1.24)	1.26 (1.13,1.42)	1.36 (1.21,1.53)	46.22	<0.001

Ranges (minimum – maximum) and odds ratios with 95% confidence intervals are given for each quintile.

Model 1 includes age, smoking, fasting status, use of cholesterol lowering medication, trial treatment assignment, hormone use, menopausal status, race, exercise, alcohol use, body mass index, diabetes, education, vegetable, fruit, sodium, and total grain intake.

Model 2 adds C-reactive protein, homocysteine, fibrinogen, soluble inter-cellular adhesion molecule 1, and hemoglobin A1C to Model 1