

Lipoprotein Subclass Abnormalities and Incident Hypertension in Initially Healthy Women

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BACKGROUND: Abnormalities in traditional lipids, particularly decreased HDL cholesterol and increased triglycerides, can precede the onset of hypertension. Whether lipoprotein particle size or subclass concentrations play a role in the development of hypertension is unknown.

METHODS: We followed 17 527 initially healthy women without baseline hypertension prospectively for 8 years. At baseline, information regarding traditional lipids and hypertension risk factors was 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. Although LDL cholesterol was not associated with hypertension [odds ratio (OR) for quintile 5 vs 1: 1.08 (95% CI 0.96–1.20)], increased concentrations of LDL particles were 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 concentrations of HDL particles had greater risk [OR 1.48 (1.32–1.67)], especially small HDL particles [OR 1.36 (1.22–1.53)], whereas large HDL particles had lower risk [OR 0.80 (0.71–0.90)]. Triglycerides and triglyceride-rich 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 compared to 0.676; *P* < 0.001).

CONCLUSIONS: In this study of initially healthy women, lipoprotein particle size and subclass concentrations were associated with incident hypertension and pro-

vided additive information to traditional lipids and risk factors.

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Hypertension is a major risk factor for cardiovascular disease and affects approximately 1 in 3 adults in the US, 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). Results of previous studies have demonstrated an increased risk of hypertension with lower concentrations of HDL cholesterol and higher concentrations of LDL cholesterol and triglycerides (3–6).

Lipoprotein abnormalities, particularly smaller LDL size and 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 that is not detected by traditional lipid testing but can be assessed by using advanced lipoprotein testing with techniques such as nuclear magnetic resonance (NMR)⁵ spectroscopy of plasma (11). NMR spectroscopy allows the simultaneous determination of both the concentration and size of lipoprotein particles, properties associated 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

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⁵ Nonstandard abbreviations: NMR, nuclear magnetic resonance; WHS, Women's Health Study; IDL, intermediate-density lipoprotein; apoB, apolipoprotein B100; BMI, body mass index; OR, odds ratio; LR, likelihood ratio.

size and concentration of LDL, HDL, and VLDL particles, and how these measures compared with those for traditional lipids and apolipoproteins. We further examined whether the association could be attenuated by biomarkers of inflammation/endothelial function, hyperglycemia, and 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 US 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 WHS participants (71%) 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 >140 mmHg, a diastolic blood pressure >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) (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 VLDL, further subdivided into large, medium, and small particles. The IDL particles, sized between VLDL and LDL particles, were categorized with the LDL particles because they exhibit similar properties. Relative mass percentages were multiplied by the diameter of each subclass to obtain weighted mean size for each lipoprotein particle (18, 19). The NMR lipoprotein variables that we examined were those that are provided when ordering a commercially available NMR lipoprotein profile (20). Particle diameters and

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/CDC Lipid Standardization Program. Traditional lipid measures used in this study (total, HDL, and LDL cholesterol and triglycerides) were all measured directly with a Hitachi 917 analyzer by using reagents from Roche Diagnostics with between-run CVs $<3\%$. We measured apolipoproteins B100 (apoB) and apoA-1 by using immunoturbidimetric assays (DiaSorin) 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 <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 with an annual questionnaire by 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 subsample of participants by 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 A_{1c} concentrations, 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 quin-

Table 1. Baseline Characteristics of 17 572 Women Initially Free of Hypertension.^a

	Incident hypertension at 8 years (n = 4714)	No hypertension at 8 years (n = 12 858)	<i>p</i> ^b
Age, years	53.6 (49.4,59.6)	51.6 (48.2,56.6)	<0.001
Systolic blood pressure, mmHg	125 (115,135)	115 (105,125)	<0.001
Diastolic blood pressure, mmHg	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	
BMI, 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
apoB, 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
apoA-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
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

Continued on page 1181

Table 1. Baseline Characteristics of 17 572 Women Initially Free of Hypertension.^a (Continued from page 1180)

	Incident hypertension at 8 years (n = 4714)	No hypertension at 8 years (n = 12 858)	<i>p</i> ^b
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
Mean NMR VLDL particle size, nm	47.6 (42.9,53)	45.5 (41.6,50.7)	<0.001

^a Values shown are median (25th percentile, 75th percentile) unless otherwise indicated.
^b Kruskal–Wallis for continuous variables, χ^2 for categorical.

tiles and were analyzed both categorically and for linear trend across quintiles using quintile number. The primary adjustment for confounding included nonlipid 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, and vegetable, fruit, sodium, and total grain intake). The increase in the likelihood ratio obtained by adding the lipid measurement to the nonlipid risk factors was also derived.

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

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 A_{1c} levels, and baseline blood pressure.

All analyses were also redone in designated subgroups and tested for interaction: (a) BMI divided into obese (BMI ≥ 30), overweight (BMI 25–30), and normal (BMI <25) (24); (b) blood pressure <120/70 mmHg; (c) metabolic syndrome categories previously used in the WHS (25) with and without the blood pressure criterion of $\geq 130/85$ mmHg; and (d) nonusers of lipid-lowering medication.

All analyses were done by using R version 2.10.1 (R Foundation for Statistical Computing).

Results

During 8 years of follow-up, incident hypertension occurred in 4714 study participants (27%). 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 concentrations of large LDL and HDL particles, and the mean 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 mean 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 nonlipid 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, and 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 mean LDL particle size were inversely associated with risk of hypertension,

Table 2. Association of LDL Measures with Incident Hypertension.^a

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	LR χ^2	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 (0.96,1.20)	3.32	0.13
apoB, 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)	1.30 (1.16,1.46)	1.35 (1.20,1.51)	44.52	<0.001
Model 2	1	1.01 (0.90,1.14)	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
Model 2	1	0.88 (0.79,0.98)	0.81 (0.73,0.89)	0.75 (0.67,0.83)	0.68 (0.60,0.76)	51.50	<0.001

^a Ranges (minimum–maximum) and ORs with 95% CIs 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, BMI, diabetes, education, and vegetable, fruit, sodium, and total grain intake. Model 2 adds C-reactive protein, homocysteine, fibrinogen, soluble intercellular adhesion molecule-1, and hemoglobin A_{1c} to model 1.

whereas increased small LDL, IDL, and hence total concentration of LDL particles, were associated with increased risk. Of note, the largest odds ratios (ORs) and likelihood ratios (LRs) 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 A_{1c} (model 2 results, Table 2).

HDL MEASURES

All HDL measures were associated with incident hypertension in unadjusted analyses (Table 3). After adjustment for nonlipid risk factors (model 1), apoA-1 was no longer independently associated, whereas standard HDL cholesterol and all HDL NMR measures remained significantly associated. The total concentration of HDL particles, specifically the medium and

Table 3. Association of HDL Measures with Incident Hypertension.^a

	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 (0.71,0.90)	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)	18.88	<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 (0.55,0.70)	66.16	<0.001
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

^a 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, BMI, diabetes, education, vegetable, fruit, sodium, and total grain intake. Model 2 adds C-reactive protein, homocysteine, fibrinogen, soluble intercellular adhesion molecule 1, and hemoglobin A_{1c} to model 1.

small HDL particles which make up most of the total concentration of HDL, were associated with increased risk, whereas large HDL particles were inversely associated with risk of hypertension. Accordingly, larger mean 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 A_{1c} 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 before adjustment, and all except small VLDL particle concentration remained associated after adjustment for nonlipid 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 asso-

Table 4. Association of VLDL and Triglyceride Measures with Incident Hypertension.^a

	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

^a 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, BMI, diabetes, education, vegetable, fruit, sodium, and total grain intake. Model 2 adds C-reactive protein, homocysteine, fibrinogen, soluble intercellular adhesion molecule 1, and hemoglobin A_{1c} to model 1.

ciated 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 A_{1c} (model 2 results, Table 4).

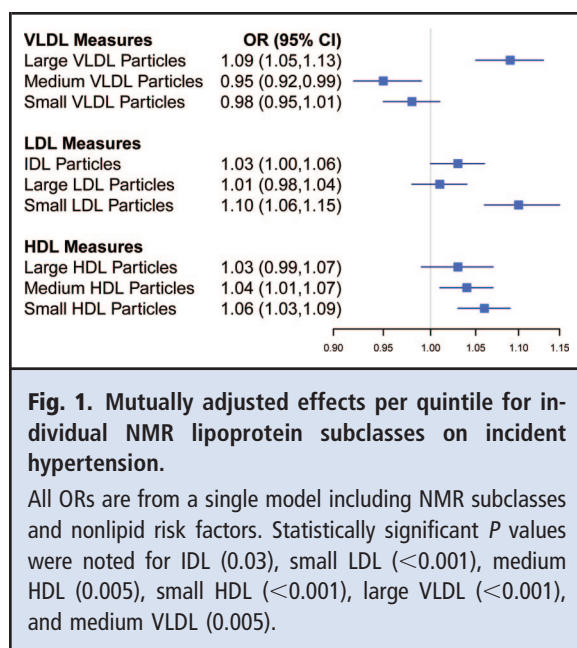
MUTUALLY ADJUSTED EFFECTS OF LIPOPROTEIN PARTICLE SUBCLASSES

In a model including quintiles of NMR particle concentrations for the 9 nonoverlapping particle subclasses and nonlipid risk factors, the medium and small HDL particles, the IDL and small LDL particles, and the medium and large VLDL particles re-

mained independently associated with hypertension risk (Fig. 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 VS SIZE

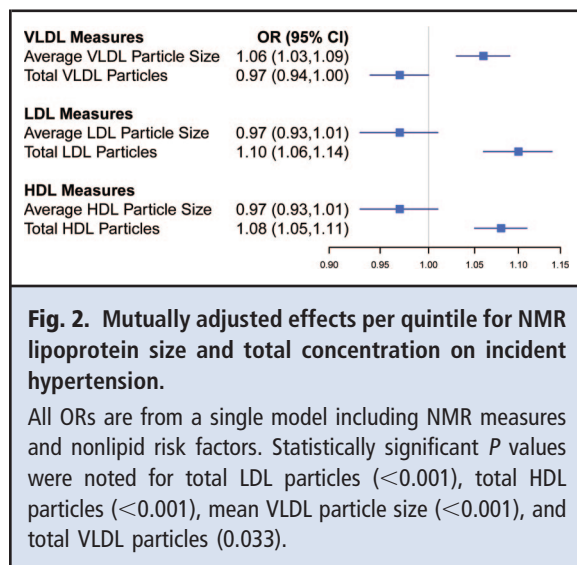
When the total particle concentration and mean particle size for each lipoprotein type were combined into one model with nonlipid risk factors, the total concentration of LDL and HDL particles and mean VLDL particle size were each independently associated with increased risk of hypertension (Fig. 2). 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 9 particle subclasses improved discrimination over a base model with nonlipid 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 mean particle size for each lipoprotein type over a base model with nonlipid 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 prespecified subgroup analysis (BMI categories, low baseline blood pressure, presence of metabolic syndrome, and nonusers of cholesterol-lowering medications). In addition, there was no evidence of interactions with any subgroup. The BMI subgroup results are shown in Supplemental Table 1 in the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol57/issue8>. 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 is 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). In addition, we found that increased total concentrations of LDL, HDL, and VLDL particles were significantly associated with increased risk, and these measures provided additional information over that provided by 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 C-reactive protein, fibrinogen, homocysteine, and soluble intercellular adhesion molecule-1, because 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 A_{1c} 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 cohort of middle-aged and older 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 benefitted from a large sample size with well-characterized study participants and a long follow-up (8 years). However, because our study was limited to women, the generalizability of our results to men remains unclear, although traditional lipids have been found to be associated with hypertension in both groups. In addition, our measure of hypertension was self-reported. Although this method may have introduced variability into the outcome measure, we believe this variability was unlikely to be related to lipoprotein measures and was therefore unlikely to have affected the direction of our results. The large sample size of the study was also helpful in providing sufficient power to enable us to observe associations despite measurement variability. Self-reported hypertension has been shown to be a valid and reliable measure in this group (28), as well as in 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 hyperten-

sion was associated with higher total concentrations of LDL and HDL particles, especially small particles, and higher total concentration of VLDL particles, especially large particles. In addition, 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 by 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.

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References

1. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart disease and stroke statistics—2011 update: a report from the American Heart Association. *Circulation* 2011; 123:e18–209.
2. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988;37: 1595–607.
3. Sesso HD, Buring JE, Chown MJ, Ridker PM, Gaziano JM. A prospective study of plasma lipid levels and hypertension in women. *Arch Intern Med* 2005;165:2420–7.
4. Halperin RO, Sesso HD, Ma J, Buring JE, Stampfer MJ, Michael Gaziano J. Dyslipidemia and the risk of incident hypertension in men. *Hypertension* 2006;47:45–50.
5. de Simone G, Devereux RB, Chinali M, Roman MJ, Best LG, Welty TK, et al. Risk factors for arterial hypertension in adults with initial optimal blood pressure: the strong heart study. *Hypertension* 2006;47:162–7.
6. Laaksonen DE, Niskanen L, Nyyssonen K, Lakka TA, Laukkanen JA, Salonen JT. Dyslipidaemia as a predictor of hypertension in middle-aged men. *Eur Heart J* 2008;29:2561–8.
7. Han SH, Quon MJ, Koh KK. Reciprocal relationships between abnormal metabolic parameters

- and endothelial dysfunction. *Curr Opin Lipidol* 2007;18:58–65.
8. Sacks FM, Campos H. Clinical review 163: cardiovascular endocrinology: low-density lipoprotein size and cardiovascular disease: a reappraisal. *J Clin Endocr Metab* 2003;88:4525–32.
 9. Urbina EM, Srinivasan SR, Kietlyka RL, Tang R, Bond MG, Chen W, Berenson GS. Correlates of carotid artery stiffness in young adults: the Bogalusa Heart Study. *Atherosclerosis* 2004;176:157–64.
 10. Sesso HD, Buring JE, Rifai N, Blake GJ, Gaziano JM, Ridker PM. C-reactive protein and the risk of developing hypertension. *JAMA* 2003;290:2945–51.
 11. Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes* 2003;52:453–62.
 12. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation* 2009;119:931–9.
 13. Ip S, Lichtenstein AH, Chung M, Lau J, Balk EM. Systematic review: association of low-density lipoprotein subfractions with cardiovascular outcomes. *Ann Intern Med* 2009;150:474–84.
 14. Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PW, et al. Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham heart study. *Circulation* 2006;113:20–9.
 15. Mora S, Otvos JD, Rosenson RS, Pradhan A, Buring JE, Ridker PM. Lipoprotein particle size and concentration by nuclear magnetic resonance and incident type 2 diabetes in women. *Diabetes* 2010;59:1153–60.
 16. Rexrode KM, Lee I, Cook NR, Hennekens CH, Buring JE. Baseline characteristics of participants in the women's health study. *J Women Health Gen-B* 2000;9:19–27.
 17. Ridker PM, Cook NR, Lee IM, Gordon D, Gaziano JM, Manson JE, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. *New Engl J Med* 2005;352:1293–304.
 18. Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. *Clin Lab* 2002;48:171–80.
 19. Otvos JD, Jeyarajah EJ, Cromwell WC. Measurement issues related to lipoprotein heterogeneity. *Am J Cardiol* 2002;90:22i–9i.
 20. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med* 2006;26:847–70.
 21. Sesso HD, Wang L, Bowman TS, Buring JE, Gaziano JM. The accuracy of self-reported hypertension in middle-aged and older women and men [Abstract]. *Circulation* 2007;115:e254.
 22. Mora S, Lee IM, Buring JE, Ridker PM. Association of physical activity and body mass index with novel and traditional cardiovascular biomarkers in women. *JAMA* 2006;295:1412–9.
 23. Rosner B, Glynn RJ. Power and sample size estimation for the Wilcoxon rank sum test with application to comparisons of c statistics from alternative prediction models. *Biometrics* 2009;65:188–97.
 24. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert panel on the identification, evaluation, and treatment of overweight in adults. *Am J Clin Nutr* 1998;68:899–917.
 25. Conen D, Rexrode KM, Creager MA, Ridker PM, Pradhan AD. Metabolic syndrome, inflammation, and risk of symptomatic peripheral artery disease in women: a prospective study. *Circulation* 2009;120:1041–7.
 26. Sattar N, Petrie JR, Jaap AJ. The atherogenic lipoprotein phenotype and vascular endothelial dysfunction. *Atherosclerosis* 1998;138:229–35.
 27. Wang TJ, Gona P, Larson MG, Levy D, Benjamin EJ, Tofler GH, et al. Multiple biomarkers and the risk of incident hypertension. *Hypertension* 2007;49:432–8.
 28. Conen D, Ridker PM, Buring JE, Glynn RJ. Risk of cardiovascular events among women with high normal blood pressure or blood pressure progression: prospective cohort study. *BMJ* 2007;335:432.
 29. Colditz GA, Martin P, Stampfer MJ, Willett WC, Sampson L, Rosner B, et al. Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. *Am J Epidemiol* 1986;123:894–900.