

SUPPLEMENTAL MATERIAL

Supplemental Methods

Laboratory Measurements

Assessment for total cholesterol used an enzymatic procedure (cholesterol esterase) with a colorimetric endpoint. Triglycerides were measured with an enzymatic hydrolysis procedure to obtain a colorimetric endpoint triglyceride value. HDL-c was measured in the resulting supernatant after heparin–manganese precipitation of apolipoprotein B–containing proteins. LDL-c was calculated by the Friedewald equation when triglycerides were <400 mg/dL,¹ and measured by ultracentrifugation when triglycerides were ≥400 mg/dL. Concentrations of whole plasma apoB and A-I were measured by immunonephelometry using a Behring nephelometric assay (Marburg, Germany). A high-sensitivity assay (Behring Nephelometer) was used for measurement of hsCRP.

Prior to ion mobility (IM) fractionation, lipoproteins were isolated by dextran sulfate precipitation. Plasma was treated with 17% ethanol which removed >97% of fibrinogen, and lipoproteins were then precipitated with dextran sulfate (2 mg/mL) and calcium (0.15 M). Precipitated lipoproteins were harvested on paramagnetic particles, washed to remove free salt and proteins and then resuspended in 25 mM ammonium acetate for analysis by ion mobility. This method recovered all measureable apoB (105%), apoA-I (96%) and total cholesterol (103%). Removal of plasma proteins was assessed by the following proteins (final concentration remaining after extraction compared with original serum concentration): IgG (3%), albumin (<4%), transferrin (0%). This new isolation procedure has excellent recovery of the lipoprotein particles based on the apolipoprotein and total cholesterol recoveries. The median apoA-I/HDL-p concentration (mM) ratio was 1.4 (Table 1 and Supplemental Tables 1 and 2) for apoA-I

molecular weight 28.07 kDa, which is similar to the ratio (1.6) determined by nuclear magnetic resonance spectroscopy in the same population.²

Following isolation, the lipoproteins were fractionated and quantitated in a single scan using gas-phase electrophoresis (ion mobility) as previously described.³ The intra-assay variation was <0.8% for LDL peak particle size and <10% for HDL-p, LDL-p, IDL-p, and VLDL-p. Inter-assay variation was <0.7% for the LDL peak particle size and <13% for HDL-p, LDL-p, IDL-p and VLDL-p. Supplemental Table 1 shows the lipoprotein subfractions, their size ranges, and nomenclature.

Supplemental Table 1. Ion mobility particle size range and nomenclature

	Size Range, nm	
Non-HDL particles, nmol/L	52.00 – 19.00	
VLDL particles, nmol/L		
Total VLDL-P	52.00 – 29.60	
Large	52.00 – 42.40	
Medium	42.40 – 33.50	
Small	33.50 – 29.60	
IDL particles, nmol/L		
Total IDL-P	29.60 – 23.33	
Large	29.60 – 25.00	
Small	25.00 – 23.33	
LDL particles, nmol/L		
Total LDL-P	23.33 – 19.00	
Large		
I	23.33 – 22.46	
II a	22.46 – 22.00	
Medium	II b	22.00 – 21.41
Small	III a	21.41 – 20.82
Very small		
III b	20.82 – 20.49	
IV a	20.49 – 19.90	
IV b	19.90 – 19.00	
IV c	19.00 – 18.00	
HDL particles, μ mol/L		
Total HDL-P	14.50 – 7.65	
Large	14.50 – 10.50	
Small	10.50 – 7.65	

Supplemental Table 2. Baseline characteristics

	Current study N=11,186	Not in current study N=6,616	Overall study N=17,802
Characteristic	Median (25 th – 75 th %ile) or N (%)	Median (25 th – 75 th %ile) or N (%)	Median (25 th – 75 th %ile) or N (%)
Age	66 (60 – 71)	66 (60 – 71)	66 (60 – 71)
Women	4,160 (37.2%)	2,641 (39.9%)	6801 (38.2%)
Rosuvastatin	5,586 (49.9%)	3,315 (50.1%)	8901 (50.0%)
Race/ethnicity			
White	8,956 (80.1%)	3,727 (56.3%)	12,683 (71.3%)
Black	963 (8.6%)	1,261 (19.1%)	2,224 (12.5%)
Asian	171 (1.5%)	112 (1.7%)	283 (1.6%)
Hispanic	992 (8.9%)	1,269 (19.2%)	2,261 (12.7%)
Other/unknown	102 (0.9%)	247 (3.7%)	349 (2.0%)
Body-mass index, kg/m ²	28.3 (25.3 – 31.9)	28.4 (25.2 – 32.1)	28.4 (25.3 – 32.0)
Systolic blood pressure, mm Hg	134 (124 – 146)	134 (125 – 145)	134 (124 – 145)
Diastolic blood pressure, mm Hg	80 (75 – 86)	80 (75 – 88)	80 (75 – 87)
Current smoker	1,746 (15.6%)	1,074 (16.2%)	2,820 (15.9%)
Family history of premature coronary disease	1,388 (12.5%)	657 (10.0%)	2,045 (11.5%)
Glucose, mg/dL	94 (88 – 101)	94 (87 – 102)	94 (88 – 102)
High-sensitivity C-reactive protein, mg/L	4.2 (2.8 – 6.9)	4.5 (3.0 – 7.5)	4.3 (2.9 – 7.1)
LDL cholesterol, mg/dL	109 (94 – 119)	108 (93 – 119)	108 (94 – 119)
Non-HDL cholesterol, mg/dL	134 (118 – 147)	135 (118 – 148)	134 (118-147)
Apolipoprotein B, mg/dL	109 (95 – 122)	110 (96 –123)	109 (95-122)
Triglycerides, mg/dL	116 (84 – 165)	122 (89 – 176)	118 (85 – 169)
HDL cholesterol, mg/dL	50 (41 – 60)	47 (39 – 58)	49 (40 – 60)
Apolipoprotein A-I, mg/dL	164 (145 – 185)	159 (141 – 181)	162 (144 – 184)

Percentages may not add up due to rounding off

Supplemental Table 3. Spearman correlation coefficients at baseline

	LDL-C	Non-HDL-C	ApoB	Trig	HDL-C	ApoA-I	Non-HDL-P	VLDL-P, total	VLDL-L	VLDL- M	VLDL- S	IDL-P, total	IDL-L	IDL-S
LDL-C	1.0													
Non-HDL-C	0.77	1.0												
ApoB	0.53	0.72	1.0											
Trig	0.05	0.61	0.48	1.0										
HDL-C	0.03	- 0.27	- 0.29	- 0.49	1.0									
ApoA-I	0.04	- 0.13	- 0.10	- 0.25	0.80	1.0								
Non-HDL-P	0.26	0.31	0.38	0.17	- 0.13	- 0.05	1.0							
VLDL-P, total	0.24	0.26	0.30	0.13	- 0.01	0.07	0.75	1.0						
VLDL-L	0.19	0.35	0.36	0.35	- 0.21	- 0.09	0.63	0.83	1.0					
VLDL-M	0.23	0.30	0.34	0.21	- 0.09	0.004	0.72	0.98	0.88	1.0				
VLDL-S	0.24	0.19	0.23	0.02	0.10	0.16	0.74	0.97	0.69	0.90	1.0			
IDL-P, total	0.26	0.16	0.18	- 0.07	0.18	0.20	0.82	0.84	0.56	0.76	0.90	1.0		
IDL-L	0.26	0.27	0.35	0.13	- 0.06	0.02	0.84	0.88	0.68	0.85	0.88	0.86	1.0	
IDL-S	0.21	0.02	- 0.01	- 0.23	0.36	0.31	0.61	0.64	0.35	0.54	0.72	0.89	0.64	1.0
LDL-P, total	0.24	0.32	0.41	0.22	- 0.21	- 0.14	0.98	0.63	0.57	0.61	0.60	0.69	0.76	0.48
LDL-I	0.25	- 0.04	- 0.03	- 0.37	0.38	0.29	0.57	0.51	0.23	0.42	0.61	0.77	0.51	0.85
LDL-IIa	0.29	0.12	0.17	- 0.13	0.08	0.06	0.73	0.53	0.36	0.48	0.57	0.69	0.61	0.62
LDL-IIb	0.24	0.31	0.41	0.24	- 0.29	- 0.21	0.78	0.46	0.46	0.47	0.43	0.47	0.65	0.29
LDL-IIIa	0.12	0.38	0.48	0.46	- 0.47	- 0.33	0.68	0.34	0.45	0.39	0.26	0.26	0.52	0.03
LDL-IIIb	0.06	0.32	0.41	0.43	- 0.41	- 0.28	0.64	0.32	0.43	0.37	0.24	0.26	0.52	0.03
LDL- IVa	0.04	0.26	0.33	0.33	- 0.31	- 0.20	0.69	0.39	0.44	0.42	0.32	0.35	0.49	0.13
LDL- IVb	0.04	0.20	0.24	0.24	- 0.17	- 0.10	0.70	0.46	0.45	0.48	0.42	0.46	0.56	0.27
LDL-IVc	0.03	0.13	0.13	0.17	- 0.05	0.01	0.66	0.53	0.46	0.52	0.51	0.57	0.58	0.42
LDL peak	0.06	- 0.32	- 0.37	- 0.60	0.59	0.41	- 0.12	0.04	- 0.21	- 0.06	0.16	0.26	- 0.05	0.49
HDL-P, total	0.04	0.02	- 0.02	- 0.03	0.16	0.16	0.65	0.48	0.35	0.43	0.52	0.63	0.51	0.58
HDL-L	0.002	- 0.09	- 0.11	- 0.15	0.31	0.29	0.57	0.51	0.29	0.43	0.59	0.68	0.51	0.65
HDL-S	0.06	0.06	0.02	0.03	0.09	0.10	0.65	0.45	0.36	0.41	0.47	0.59	0.48	0.52

Supplemental Table 3. Spearman correlation coefficients at baseline, continued

	LDL-P, total	LDL-I	LDL-IIa	LDL-IIb	LDL-IIIa	LDL-IIIb	LDL-IVa	LDL-IVb	LDL-IVc	LDL peak	HDL-P, total	HDL-L	HDL-S
LDL-P, total	1.0												
LDL-I	0.47	1.0											
LDL-IIa	0.70	0.83	1.0										
LDL-IIb	0.84	0.33	0.73	1.0									
LDL-IIIa	0.78	-0.08	0.28	0.80	1.0								
LDL-IIIb	0.72	-0.12	0.14	0.62	0.93	1.0							
LDL-IVa	0.75	0.01	0.18	0.52	0.80	0.92	1.0						
LDL-IVb	0.72	0.14	0.26	0.45	0.61	0.73	0.88	1.0					
LDL-IVc	0.64	0.25	0.29	0.35	0.42	0.50	0.65	0.86	1.0				
LDL peak	-0.24	0.64	0.26	-0.33	-0.67	-0.64	-0.50	-0.32	-0.14	1.0			
HDL-P, total	0.61	0.55	0.54	0.43	0.30	0.30	0.39	0.43	0.49	0.12	1.0		
HDL-L	0.50	0.60	0.50	0.30	0.13	0.15	0.27	0.37	0.48	0.27	0.91	1.0	
HDL-S	0.62	0.50	0.54	0.46	0.35	0.34	0.41	0.43	0.47	0.05	0.99	0.83	1.0

Supplemental Table 4. Baseline and on-treatment LDL subfractions (in clinical categories) in relation to incident CVD events

	CVD HR per SD higher* (95% CI)	P	CVD & all-cause death HR per SD higher* (95% CI)	P
Placebo, baseline				
LDL particles, nmol/L				
Large, I-IIa	1.08 (0.93-1.24)	0.32 ‡	0.96 (0.85-1.08)	0.51
Medium, IIb	1.22 (1.08-1.39)	0.002 ‡	1.06 (0.95-1.18)	0.30
Small, IIIa	1.32 (1.13-1.53)	<0.001 ‡	1.15 (1.02-1.30)	0.018
Very small, IIIb-IVc	1.24 (1.07-1.42)	0.003	1.21 (1.09-1.35)	<0.001 ‡
Rosuvastatin, on-treatment				
LDL particles, nmol/L				
Large, I-IIa	1.21 (0.89-1.66)	0.23	1.30 (1.01-1.66)	0.040
Medium, IIb	1.12 (0.85-1.49)	0.42	1.31 (1.03-1.66)	0.029
Small, IIIa	1.13 (0.86-1.48)	0.37	1.25 (1.00-1.57)	0.050
Very small, IIIb-IVc	0.94 (0.72-1.22)	0.64	1.06 (0.84-1.34)	0.60

Abbreviations: HR, hazard ratio from Cox proportional hazard model; CI: confidence interval

* Per 1-SD increment in the LDL subfraction, adjusted for age, sex, race, smoking, family history, BMI, systolic blood pressure, glucose, ln hsCRP, and randomized treatment assignment (rosuvastatin vs placebo). SDs were (nmol/L): large (264), medium (129), small (151), very small (235). Small and very small LDL subfractions were natural log transformed; values shown are the untransformed SDs.

‡ All measures shown in this table were additionally evaluated in models that included LDL cholesterol, HDL cholesterol, ln triglycerides in addition to age, sex, race, smoking, family history, BMI, systolic blood pressure, glucose, and ln hsCRP, with variables that met statistical significance ($P < 0.05$) indicated by ‡. For CVD end point: LDL Large HR = 1.18 (95% CI, 1.02-1.37), $P = 0.027$; LDL Medium HR = 1.18 (95%CI, 1.03-1.35), $P = 0.018$; LDL Small HR = 1.20 (95%CI, 1.01-1.42), $P = 0.040$. For CVD and all cause death end point: LDL Very small HR = 1.16 (95%CI, 1.03-1.31), $P = 0.012$.

Supplemental Table 5. Baseline lipid and lipoprotein examined as top vs bottom tertiles in relation to incident CVD events among the placebo arm

	CVD		CVD & all-cause death	
	HR Top vs Bottom Tertile* (95% CI)	P	HR Top vs Bottom Tertile* (95% CI)	P
Lipids and apolipoproteins				
LDL cholesterol	1.16 (0.82-1.62)	0.40	0.89 (0.68-1.16)	0.40
Non-HDL cholesterol	1.38 (0.99-1.93)	0.061	1.07 (0.82-1.40)	0.63
Apolipoprotein B	1.50 (1.05-2.14)	0.026	1.03 (0.78-1.36)	0.84
Triglycerides	1.85 (1.29-2.65)	0.001 ‡	1.50 (1.14-1.99)	0.004
HDL cholesterol	0.54 (0.37-0.79)	0.001 ‡	0.59 (0.44-0.80)	0.001 ‡
Apolipoprotein A-I	0.50 (0.34-0.73)	<0.001 ‡	0.53 (0.39-0.72)	<0.001 ‡
Ion mobility				
Non-HDL particles	1.52 (1.05-2.20)	0.026	1.04 (0.78-1.39)	0.78
VLDL particles				
Total	1.43 (1.02-2.01)	0.041	1.02 (0.78-1.35)	0.87
Large	1.54 (1.09-2.17)	0.014	1.25 (0.96-1.63)	0.10
Medium	1.38 (0.98-1.93)	0.061	1.06 (0.81-1.38)	0.70
Small	1.20 (0.84-1.71)	0.32	0.81 (0.60-1.08)	0.15
IDL particles				
Total	1.21 (0.85-1.72)	0.29 ‡	0.97 (0.73-1.29)	0.84
Large	1.25 (0.88-1.76)	0.21	0.91 (0.69-1.21)	0.53
Small	1.33 (0.95-1.88)	0.10 ‡	1.21 (0.91-1.60)	0.19 ‡
LDL particles				
Total	1.65 (1.14-2.40)	0.009 ‡	1.19 (0.90-1.58)	0.23
I (largest)	0.92 (0.66-1.29)	0.63	0.86 (0.65-1.13)	0.28
II a	1.14 (0.82-1.61)	0.43	0.94 (0.71-1.23)	0.63
II b	1.72 (1.18-2.51)	0.005 ‡	1.13 (0.85-1.51)	0.41
III a	1.82 (1.26-2.65)	0.002	1.38 (1.03-1.86)	0.030
III b	1.45 (1.00-2.10)	0.050	1.22 (0.91-1.63)	0.18

IV a	1.37 (0.93-2.01)	0.12	1.22 (0.91-1.63)	0.19
IV b	1.50 (1.02-2.19)	0.039	1.59 (1.19-2.13)	0.002 ‡
IV c (smallest)	2.00 (1.39-2.88)	<0.001 ‡	2.41 (1.81-3.20)	<0.001 ‡
LDL peak diameter	0.60 (0.42-0.87)	0.007	0.76 (0.57-1.01)	0.056
HDL particles				
Total	1.22 (0.85-1.77)	0.28	1.12 (0.84-1.50)	0.45
Large	1.02 (0.72-1.45)	0.91	1.02 (0.77-1.35)	0.90
Small	1.29 (0.89-1.87)	0.18	1.16 (0.86-1.55)	0.32

Abbreviations: HR, hazard ratio from Cox proportional hazard model; CI: confidence interval

*Comparing top versus bottom tertile in the lipid or lipoprotein variable, adjusted for age, sex, race, smoking, family history, BMI, systolic blood pressure, glucose, ln hsCRP, and randomized treatment assignment (rosuvastatin vs placebo)

‡ All measures shown in this table were additionally evaluated in models that included LDL cholesterol, HDL cholesterol, ln triglycerides in addition to age, sex, race, smoking, family history, BMI, systolic blood pressure, glucose, and ln hsCRP, with variables that met statistical significance ($P < 0.05$) indicated by ‡.

Supplemental Table 6. On-treatment lipid and lipoprotein examined as top vs bottom tertiles in relation to incident CVD events among the rosuvastatin arm

	CVD		CVD & all-cause death	
	HR Top vs Bottom Tertile* (95% CI)	P	HR Top vs Bottom Tertile* (95% CI)	P
Lipids and apolipoproteins				
LDL cholesterol	1.98 (1.18-3.34)	0.010	1.56 (1.01-2.40)	0.045
Non-HDL cholesterol	1.86 (1.07-3.21)	0.027	1.89 (1.20-2.99)	0.006
Apolipoprotein B	2.06 (1.17-3.63)	0.012	2.03 (1.27-3.25)	0.003
Triglycerides	1.14 (0.64-2.03)	0.66	1.19 (0.74-1.92)	0.47
HDL cholesterol	0.64 (0.33-1.22)	0.17	0.57 (0.34-0.96)	0.036
Apolipoprotein A-I	0.83 (0.45-1.55)	0.56	0.61 (0.36-1.02)	0.059
Ion mobility				
Non-HDL particles	1.07 (0.59-1.93)	0.83	1.26 (0.77-2.07)	0.35
VLDL particles				
Total	1.23 (0.71-2.14)	0.47	1.31 (0.83-2.07)	0.24
Large	0.96 (0.56-1.65)	0.90	1.16 (0.73-1.84)	0.53
Medium	1.08 (0.62-1.89)	0.77	1.38 (0.87-2.20)	0.18
Small	1.48 (0.85-2.56)	0.16	1.47 (0.94-2.31)	0.093
IDL particles				
Total	1.44 (0.81-2.58)	0.22	1.43 (0.89-2.32)	0.14
Large	1.39 (0.79-2.44)	0.25	1.33 (0.83-2.13)	0.23
Small	1.56 (0.89-2.75)	0.12	1.57 (0.97-2.54)	0.064
LDL particles				
Total	1.15 (0.65-2.03)	0.64	1.31 (0.81-2.11)	0.27
I (largest)	1.13 (0.61-2.07)	0.70	1.19 (0.73-1.94)	0.49
II a	1.22 (0.69-2.19)	0.49	1.27 (0.79-2.04)	0.32
II b	1.23 (0.71-2.13)	0.45	1.43 (0.90-2.28)	0.13
III a	1.14 (0.66-1.97)	0.64	1.29 (0.81-2.07)	0.29

III b	0.74 (0.42-1.31)	0.30	0.86 (0.53-1.40)	0.55
IV a	0.98 (0.55-1.74)	0.95	1.11 (0.68-1.81)	0.67
IV b	1.16 (0.65-2.08)	0.62	1.35 (0.84-2.18)	0.21
IV c (smallest)	1.13 (0.63-2.03)	0.69	1.37 (0.86-2.17)	0.18
LDL peak diameter	1.36 (0.76-2.48)	0.31	1.19 (0.73-1.92)	0.49
HDL particles				
Total	1.01 (0.56-1.80)	0.98	1.09 (0.67-1.77)	0.72
Large	0.74 (0.41-1.32)	0.31	0.86 (0.54-1.38)	0.54
Small	1.13 (0.64-2.00)	0.68	1.19 (0.73-1.91)	0.49

Abbreviations: HR, hazard ratio from Cox proportional hazard model; CI: confidence interval

*Comparing top versus bottom tertile in the lipid or lipoprotein variable, adjusted for age, sex, race, smoking, family history, BMI, systolic blood pressure, glucose, ln hsCRP, and randomized treatment assignment (rosuvastatin vs placebo)

A total of 73 CVD events and 108 CVD/all-cause death events occurred among 4597 individuals with complete 12-month lipid and lipoprotein data.

Supplemental Table 7. Model prediction performance of baseline and on-treatment ion mobility subfractions when added as a set to a model with established risk factors

	CVD		CVD & all-cause death	
	Likelihood Ratio χ^2 (d.f.; multivariable p value)	Discrimination c-index	Likelihood Ratio χ^2 (d.f.; multivariable p value)	Discrimination c-index
Placebo, baseline				
Risk factors alone*	Ref.	0.681	Ref.	0.701
Risk factors plus full set of IM subfractions	41.80 (d.f.=15; p=0.0002)	0.705	99.19 (d.f.=15; p<0.0001)	0.741
Risk factors plus parsimonious set of IM subfractions‡	35.32 (d.f.=6; p<0.0001)	0.703	94.23 (d.f.=8; p<0.0001)	0.736
Rosuvastatin, on-treatment				
Risk factors alone	Ref.	0.743	Ref.	0.737
Risk factors plus full set of IM subfractions	17.19 (d.f.=15; p=0.308)	0.764	20.74 (d.f.=15; p=0.145)	0.740
Risk factors plus parsimonious set of IM subfractions**	11.70 (d.f.=3; p=0.009)	0.758	15.20 (d.f.=3; p=0.002)	0.741

Abbreviations: IM, Ion mobility

*LDL cholesterol, HDL cholesterol, triglycerides, age, race, smoking, family history of premature coronary disease, BMI, systolic blood pressure, fasting glucose, and ln hsCRP.

‡ parsimonious set of IM biomarkers selected by backward elimination were:

- 1) CVD: LDL IIIa, LDL IIIb, LDL IVc, IDL large, IDL small, HDL large.
- 2) CVD & all-cause death: same as for CVD above plus VLDL large and VLDL small.

**parsimonious set of IM biomarkers selected by backward elimination were:

- 1) CVD: LDL IIb, LDL IIIa, and LDL IIIb.
- 2) CVD & all-cause death: LDL IIIa, LDL IIIb, and LDL IVa.

Supplemental Figures

Figure S1

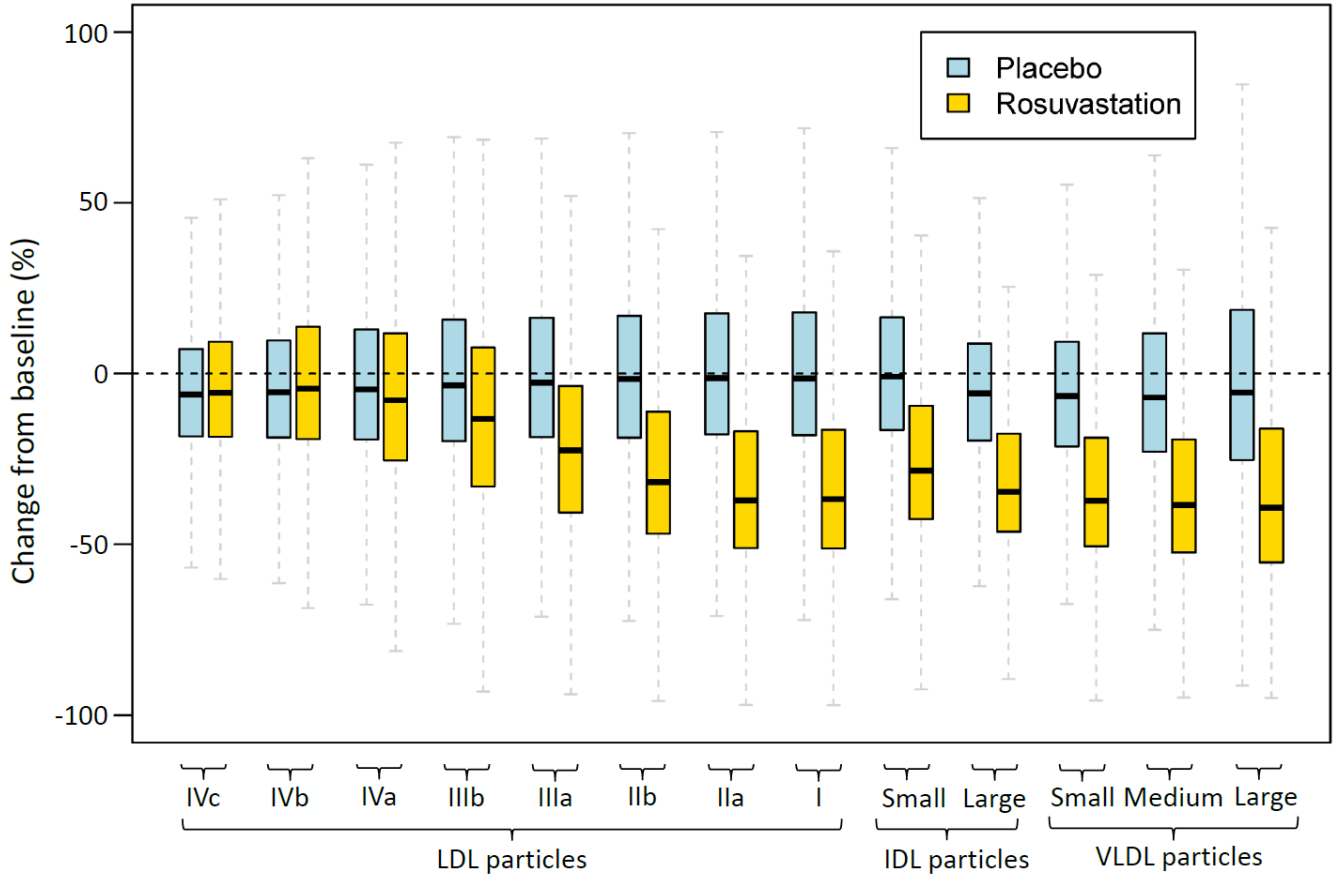
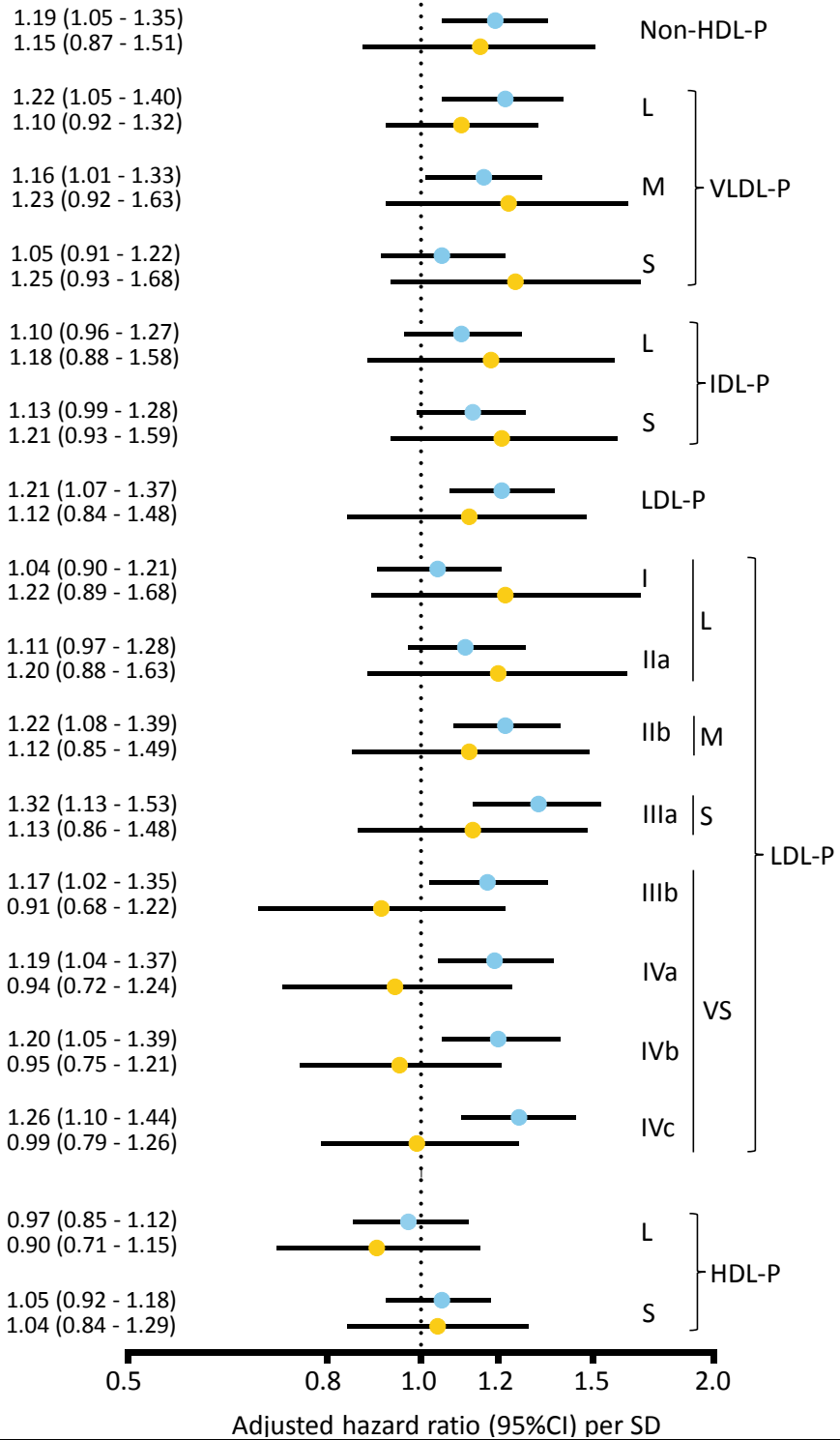


Figure S2 Adjusted hazard ratio (95%CI)



Supplemental Figure Legends

Figure S1. Changes of ion mobility subfraction concentrations from baseline according to treatment group. The boxplots show the median, 25th, and 75th percentiles. The whiskers extend 1.5 times the interquartile range. Changes in subfraction levels were calculated as $[100 \times (\text{concentration at 1 year} - \text{concentration at baseline}) / \text{concentration at baseline}]$.

Figure S2. Associations between the trial primary endpoint of cardiovascular events and lipoprotein subfractions. Subfraction levels were determined at baseline in the placebo group (blue) and at 1 year on study in the rosuvastatin group (gold). Hazard ratios were adjusted for age, sex, race, smoking, family history, body mass index, systolic blood pressure, fasting glucose, and log-transformed C-reactive protein. SD, standard deviation; CI, confidence interval.

Supplemental References

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