VLDL, Apolipoproteins B, CIII, and E, and Risk of Recurrent Coronary Events in the Cholesterol and Recurrent Events (CARE) Trial

VLDL, Apolipoproteins B, CIII, and E, and Risk of Recurrent Coronary Events in the Cholesterol and Recurrent Events (CARE) Trial

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Background—Plasma triglyceride concentration has been an inconsistent independent risk factor for coronary heart disease, perhaps because of the metabolic heterogeneity among VLDL particles, the main carriers of triglycerides in plasma.

Methods and Results—We conducted a prospective, nested case-control study in the Cholesterol and Recurrent Events (CARE) trial, a randomized placebo-controlled trial of pravastatin in 4159 patients with myocardial infarction and average LDL concentrations at baseline (115 to 174 mg/dL, mean 139 mg/dL). Baseline concentrations of VLDL–apolipoprotein (apo) B (the VLDL particle concentration), VLDL lipids, and apoCIII and apoE in VLDL+LDL and in HDL were compared in patients who had either a myocardial infarction or coronary death (cases, n=418) with those in patients who did not have a cardiovascular event (control subjects, n=370) in 5 years of follow-up. VLDL-cholesterol, VLDL-triglyceride, VLDL-apoB, apoCIII and apoE in VLDL+LDL and apoE in HDL were all interrelated, and each was a univariate predictor of subsequent coronary events. The significant independent predictors were VLDL-apoB (relative risk [RR] 3.2 for highest to lowest quintiles, $P=0.04$), apoCIII in VLDL+LDL (RR 2.3, $P=0.04$), and apoE in HDL (RR 1.8, $P=0.02$). Plasma triglycerides, a univariate predictor of coronary events (RR 1.6, $P=0.03$), was not related to coronary events (RR 1.3, $P=0.6$) when apoCIII in VLDL+LDL was included in the model, whereas apoCIII remained significant. Adjustment for LDL- and HDL-cholesterol did not affect these results.

Conclusions—The plasma concentrations of VLDL particles and apoCIII in VLDL and LDL are more specific measures of coronary heart disease risk than plasma triglycerides perhaps because their known metabolic properties link them more closely to atherosclerosis. **(*Circulation*. 2000;102:1886-1892.)**

Key Words: coronary disease ■ apolipoproteins ■ lipoproteins ■ cholesterol

Plasma triglyceride concentration is a significant predictor of coronary heart disease (CHD).[1–3] However, as an independent lipid risk factor, triglycerides is weaker than LDL-cholesterol or HDL-cholesterol. For example, in men, a 30% change in plasma lipid concentration corresponds to a change in coronary risk of 7% for triglycerides[1] versus 30% for LDL-cholesterol or HDL-cholesterol.[4,5] VLDL, the major carrier of plasma triglycerides, are a diverse group of lipoprotein particles that vary in triglyceride and cholesterol content, apolipoprotein (apo) C and apoE, and in their metabolism.[6,7] ApoCIII and apoE are major determinants of the metabolism of VLDL in plasma, and, in animal models, they accelerate[8] and protect against[9] atherosclerosis, respectively. It has been proposed that the apolipoprotein composition of lipoproteins is more closely linked to CHD than the conventional lipoprotein measurements of lipid content and density.[6] In case-control studies, apoCIII concentrations in VLDL+LDL were higher in patients with coronary disease compared with that in control subjects[10,11] and were correlated with worsening coronary stenosis on angiography.[12–14] Surprisingly, patients with CHD have higher apoE concentrations in VLDL+LDL than control subjects.[10,11,15,16] Finally, since entire lipoprotein particles enter the arterial intima, perhaps the VLDL concentration or particle size may be the most important variable.[17] The present study directly compares major constituents of VLDL and the apoE and apoCIII concentrations in VLDL+LDL as predictors of recurrent coronary events.

Methods

We used a prospective, nested, case-control design in the Cholesterol and Recurrent Events (CARE) trial. CARE was a randomized, placebo-controlled trial of pravastatin in 4159 patients who had acute

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TABLE 1. Patient Characteristics and Lipoprotein Concentrations

	Control Subjects	Cases	*P*
n	370	418	
Age, y	60±10	60±10	0.9
Men	87%	87%	0.6
Smokers	10%	22%	<0.001
Hypertension	40%	48%	0.02
Diabetic	9%	22%	<0.001
Waist circumference, cm	38±5	39±5	0.005
Body mass index, kg/m^2	27±4	29±15	0.04
Ejection fraction	54±12%	50±12%	<0.001
β-Blockers	40%	46%	0.05
Diuretics	11%	17%	0.02
Lipid variables, mg/dL			
Cholesterol	207±17	210±17	0.04
Triglycerides	150±60	159±59	0.04
VLDL-cholesterol	28 ±14	30±14	0.09
VLDL-triglyceride	100±58	108±57	0.055
VLDL-apoB	12.4±7.7	13.5±7.9	0.04
LDL-cholesterol	138±14	139±15	0.11
ApoCIII (VLDL+LDL)	6.1±2.5	6.6±2.6	0.007
ApoCIII (HDL)	5.3±1.8	5.5±2.0	0.15
ApoE (VLDL+LDL)	6.0±1.7	6.3±1.8	0.045
ApoE (HDL)	4.2±1.8	4.5±2.0	0.01
ApoAI	118±19	117±19	0.39
HDL-cholesterol	39±10	38±9	0.13
ApoB	121±17	123±16	0.15

Values are % of population except as indicated.

myocardial infarction 3 to 20 months before enrollment.[18] The institutional review board at each participating clinical center approved the study, subjects gave informed consent, and all procedures followed were in accordance with institutional guidelines. Baseline total cholesterol was <240 mg/dL, LDL-cholesterol 115 to 174 mg/dL, and triglycerides <350 mg/dL. The median follow-up duration was 5 years. During the trial, 486 patients had a primary end point, coronary death, or myocardial infarction.[18] Sufficient plasma for analysis was available from both screening visits for 418 of these "cases." The baseline characteristics of these 418 patients were similar to the 68 patients for whom sufficient plasma was not available. Patients who did not have a primary end point that matched the cases for decade of age (eg, 40 to 49, 50 to 59 years) and sex were randomly selected. Among the 418 matches, 370 did not have coronary bypass surgery, coronary angioplasty, or stroke (or a primary end point) after randomization during the follow-up and were selected as the control subjects. We excluded these 48 patients because the results of the trial demonstrated that pravastatin reduced coronary revascularization and stroke,[18] and patients who had these clinical cardiovascular end points should not be considered as event-free control subjects.

Fasting venous blood was taken from each patient on each of 2 screening visits, ≥1 week apart, and sent by overnight delivery in cooled containers to the core laboratory in St Louis, Missouri. Edetic acid was used as an anticoagulant and preservative. Plasma was separated in a refrigerated centrifuge, and 1-mL aliquots were placed in polypropylene vials and stored continuously at −80°C until analysis. After the trial concluded, vials containing frozen plasma from the 2 screening visits were shipped on solid CO_2 by overnight delivery from the core laboratory to the laboratories of Dr Alaupovic (Oklahoma Medical Research Foundation, Oklahoma City) for apoCIII and apoE measurements and of Dr Sacks (Harvard School of Public Health, Boston, Mass) for VLDL lipid and apoB measurements. The average time between collection of samples and these analyses was 8 years, with a range of 7 to 9 years. The mean concentrations and ranges of the total cholesterol, LDL-C, HDL-C and triglycerides in the present study were similar to those measured on the same patients with fresh plasma.[18] Apolipoprotein measurements have been affected minimally by long-term frozen storage (P. Alaupovic, unpublished findings). For example, mean apoCIII in VLDL+LDL in 20 patients was 2.4±0.8 mg/dL in fresh plasma versus 2.7±1.0 mg/dL after 2.5 years storage at −70°C, which included a thawing and refreezing. ApoB decreased by 4.5% (NS) during this time. Total plasma apoCIII in 44 patients was 19±7 mg/dL in fresh plasma versus 21±7 mg/dL after 5 years of continuous storage at −20°C; apo B was 137±25 versus 117±22 mg/dL ($P<0.001$). The reduction in apoB after 5 years of storage at −20°C was uniform across samples, ranging from 14% to 22% of initial values. Thus, we expect that any changes in apoB in the present study in which the samples were stored at −80°C would have been minimal. For each patient, an equal volume of plasma from the 2 screening visits was combined for analysis to reduce the influence of biological variation. Analysis was conducted with the matched cases and control subjects in each laboratory batch so that run-to-run variation would not add imprecision to the case-control differences in the measurements. All personnel at the laboratories were blinded to the case-control code, which was maintained at the Data Coordinating Center, University of Texas School of Public Health, Houston.

VLDL was prepared by ultracentrifugation of plasma overlayered with 0.9 mol/L sodium chloride. In VLDL, cholesterol, unesterified cholesterol, and triglyceride were measured by enzymic methods, and apoB by ELISA. Polyclonal anti-apoB was used for capture and detection. LDL-cholesterol was measured directly in the LDL fraction isolated by ultracentrifugation within a density range of 1.006 to 1.063 g/mL. Within-run coefficients of variation were 3% for VLDL-cholesterol, 4% for VLDL-triglycerides, 4% for VLDL-apoB, and 2% for LDL-cholesterol.

Plasma (1 mL) was precipitated with heparin manganese to remove apoB-containing lipoproteins (VLDL+LDL).[19] The supernatant containing HDL was removed, and the precipitate was dissolved in PBS containing 0.025% Tween 20. ApoCIII[20] and apoE[21] were measured in plasma, and the supernatant (HDL) and precipitate (VLDL+LDL) by immunoturbidimetry. The within-run assay coefficients of variation were 7% for apoCIII in the supernatant, 3% for apoCIII in the precipitate, 9% for apoE in the supernatant, and 7% for apoE in the precipitate.

Statistical analyses were performed at the University of Texas Public Health School. The distribution of lipid measurements of the control subjects was used to compute quintiles, and the number of cases and control subjects in each quintile were determined. Multiple logistic regression computed odds ratios for case status for the second through fifth quintiles compared with the first quintile. Tests for linear trend were performed on the relative risks across quintiles, with the median value for each of the quintiles. The primary model included covariates of age, smoking, hypertension, and left ventricular ejection fraction. In additional models, other lipid and nonlipid covariates were added as described. The primary analysis included all cases and control subjects regardless of treatment assignment in the trial to placebo or pravastatin. In additional analyses, lipoprotein variables were investigated in each treatment group separately, and tests for interaction between treatment assignment and relative risk of coronary events for a lipoprotein concentration were conducted. A probability value of 0.05 (2-sided) was considered significant.

Results

Compared with the control subjects, the cases had a higher mean body mass index and waist circumference; more of

TABLE 2. Correlations Among Lipoprotein Concentration, Body Mass Index, and Waist Circumference

	VLDL-apoB	VLDL-Cholesterol	VLDL-TG	Apo CIII-VLDL+LDL	ApoE-VLDL+LDL	Apo CIII-HDL	ApoE-HDL	LDL-Cholesterol	HDL-Cholesterol	Triglycerides
VLDL-apoB	...	0.88	0.84	0.72	0.63	0.32	0.40	−0.19	−0.43	0.80
VLDL-cholesterol	0.88	...	0.90	0.81	0.74	0.35	0.46	−0.24	−0.48	0.91
VLDL-TG	0.84	0.90	...	0.83	0.72	0.36	0.43	−0.22	−0.45	0.92
ApoCIII-VLDL+LDL	0.72	0.81	0.83	...	0.84	0.19	0.31	−0.15	−0.38	0.84
ApoE-VLDL+LDL	0.63	0.74	0.72	0.84	...	0.15	0.22	−0.10	−0.33	0.73
ApoCIII-HDL	0.32	0.35	0.36	0.19	0.15	...	0.70	−0.21	0.13	0.45
ApoE-HDL	0.40	0.46	0.43	0.31	0.22	0.70	...	−0.22	−0.11	0.52
LDL-Cholesterol	−0.19	−0.24	−0.22	−0.15	−0.10	−0.21	−0.22	...	0.11	−0.25
HDL-Cholesterol	−0.43	−0.48	−0.45	−0.38	−0.30	0.13	−0.11	0.11	—	−0.45
Triglycerides	0.80	0.91	0.92	0.84	0.73	0.45	0.52	−0.25	−0.45	...
Body mass index	0.05	0.07	0.07	0.09	0.07	−0.01	0.06	−0.04	−0.06	0.08
Waist circumference	0.14	0.18	0.21	0.19	0.15	0.03	0.13	−0.07	−0.24	0.21

n=788.
Values are coefficients of correlation (Pearson test).
Values of 0.065 to 0.10, $P<0.01$; ≥0.11, $P<0.001$.

them were current smokers, hypertensive, or diabetic, and more used β-blockers and diuretics (Table 1).

Triglycerides, VLDL-apoB, VLDL-cholesterol, VLDL-triglycerides, and apoCIII and apoE in VLDL+LDL were strongly correlated with one another ($r=0.63$ to 0.92), moderately inversely correlated with HDL cholesterol ($r=-0.33$ to -0.48), and weakly inversely correlated with LDL cholesterol ($r=-0.10$ to -0.24) (Table 2). Triglycerides, VLDL-apoB, VLDL-cholesterol, and VLDL-triglycerides were moderately correlated with apoE and apoCIII in HDL ($r=0.32$ to 0.52).

VLDL-apoB, VLDL-cholesterol, and VLDL-triglycerides were predictors of recurrent coronary events, comparing the highest and lowest quintiles, and the test for linear trend was significant for VLDL-apoB and VLDL-triglycerides (Table 3). The VLDL measurements were studied together in multiple logistic regression to determine which were independent. VLDL-apoB was the strongest predictor of recurrent events (relative risk [RR] 3.2, $P=0.04$) for the highest compared with the lowest quintile, whereas VLDL-cholesterol trended toward an inverse relation with simultaneous adjustment ($P=0.10$) (Figure 1). In this model, LDL-cholesterol remained a significant predictor (RR 1.7, $P=0.03$), whereas HDL-cholesterol (RR 0.97) and triglycerides (RR 1.8, $P=0.25$) were not significant. When VLDL-triglyceride was substituted for total triglyceride, the results were similar. VLDL-cholesterol ester and VLDL-unesterified cholesterol had a similar relation to coronary events as VLDL-cholesterol (cholesterol ester+unesterified cholesterol) (data not shown). The number of triglyceride molecules in VLDL, the major determinant of VLDL size, was not associated with coronary events (Table 3).

ApoCIII in VLDL+LDL was a strong significant predictor of coronary events (RR 2.25, $P=0.001$), whereas apoE in VLDL+LDL was less so (RR 1.68, $P=0.03$) (Table 3). When both were included in the logistic regression model, apoCIII remained a significant predictor, whereas apoE lost its association with coronary events (Figure 2). The amount of apoCIII per VLDL/LDL particle (apoCIII/apoB ratio) was a significant predictor, whereas the apoE/apoB ratio had no relation to coronary events (Table 3). ApoCIII in VLDL+LDL (RR 2.3, $P=0.04$) and VLDL-apoB (RR 3.2, $P=0.04$) were both significant independent predictors of coronary events in a single model that included LDL cholesterol, VLDL cholesterol, triglycerides, and HDL cholesterol (Figure 3). However, the predictive value for triglycerides, significant in univariate analysis (RR 1.58, $P=0.03$) (Table 3), was lost (RR 1.3, $P=0.6$ for fifth versus first quintile) (Figure 3).

The apoE concentration in HDL significantly predicted recurrent coronary events when studied in several models (1) as a univariate lipoprotein measurement in the standard model (RR 2.05, $P=0.002$) (Table 3), (2) with LDL-cholesterol, HDL-cholesterol, and triglycerides (RR 1.9, 0.009), and (3) together with apoCIII in VLDL+LDL (RR 1.8, $P=0.02$). The significant positive univariate association between triglycerides and coronary events (Table 3) was not present when apoE in HDL was included (RR 1.2, $P=0.5$), whereas LDL-C remained significant (RR 1.8, $P=0.01$). Including apoE in VLDL+LDL did not alter the relative risk for apoE in HDL. The apoCIII concentration in HDL was not significant in any of the models.

The relative risks for the significant lipoprotein predictors were similar with or without adjustment for diabetes in multivariate analysis; the relative risks for the highest compared with the lowest quintile of VLDL-apoB was 1.85 compared with 1.95 (Table 3) with and without adjustment, respectively, for apoCIII in VLDL+LDL (RR 2.07 versus 2.25) and for apoE in HDL (RR 1.95 versus 2.05). The relative risks were also similar in an analyses that excluded the diabetic patients. For example, in the full multivariate model shown in Figure 3, the relative risk for VLDL-apoB

TABLE 3. Relative Risks of Recurrent Coronary Events for Lipoprotein Concentrations and Composition

	Quintiles					
	1	2	3	4	5	Trend
VLDL-apoB, mg/dL						
Mean	4.5	8.0	11.0	14.0	24.0	
RR	1.00	1.74	1.69	2.02	1.95	0.03
CI	...	(1.1, 2.8)	(1.0, 2.8)	(1.2, 3.3)	(1.2, 3.2)	
P	...	0.025	0.04	0.004	0.007	
VLDL-cholesterol, mg/dL						
Mean	11	19	26	34	51	
RR	1.00	1.64	1.49	1.66	1.69	0.08
CI	...	(1.0, 2.6)	(0.9, 2.4)	(1.0, 2.7)	(1.1, 2.7)	
P	...	0.04	0.10	0.04	0.03	
VLDL-TG, mg/dL						
Mean	36	64	88	121	193	
RR	1.00	1.31	1.48	1.83	1.62	0.04
CI	...	(0.8, 2.1)	(0.9, 2.4)	(1.1, 2.9)	(1.0, 2.6)	
P	...	0.30	0.11	0.01	0.045	
VLDL-cholesterol/apoB, mol/mol						
Mean	2600	3100	3300	3600	4500	
RR	1.00	1.05	1.03	0.83	0.63	0.03
CI	...	(0.7, 1.6)	(0.7, 1.6)	(0.5, 1.3)	(0.4, 1.0)	
P	...	0.8	0.9	0.4	0.06	
VLDL-TG/apoB, mol/mol						
Mean	3700	4500	5000	5600	7300	
RR	1.00	0.72	0.88	0.83	1.01	0.7
CI	...	(0.4, 1.2)	(0.6, 1.4)	(0.5, 1.3)	(0.6, 1.6)	
P	...	0.2	0.6	0.4	1.0	
ApoCIII in VLDL+LDL, mg/dL						
Mean	3.2	4.6	5.6	7.0	10.2	
RR	1.00	1.94	1.81	2.17	2.25	0.005
CI	...	(1.2, 3.2)	(1.1, 3.0)	(1.3, 3.5)	(1.4, 3.6)	
P	...	0.008	0.019	0.002	0.001	
ApoCIII/apoB in VLDL+LDL, mol/mol						
Mean	2.4	3.1	3.9	4.7	6.3	
RR	1.00	1.57	1.33	1.71	1.78	0.03
CI	...	(1.0, 2.5)	(0.8, 2.2)	(1.1, 2.8)	(1.1, 2.9)	
P	...	0.07	0.25	0.03	0.02	
ApoE in VLDL+LDL, mg/dL						
Mean	4.0	5.0	5.8	6.8	8.7	
RR	1.00	1.30	1.38	1.16	1.68	0.06
CI	...	(0.8, 2.1)	(0.9, 2.2)	(0.7, 1.9)	(1.1, 2.6)	
P	...	0.3	0.2	0.5	0.03	
ApoE/apoB in VLDL+LDL, mol/mol						
Mean	0.4	0.5	0.6	0.7	0.9	
RR	1.00	0.96	0.92	1.08	1.16	0.4
CI	...	(0.6, 1.5)	(0.6, 1.5)	(0.7, 1.8)	(0.9, 1.2)	
P	...	0.9	0.7	0.8	0.5	
ApoCIII in HDL, mg/dL						
Mean	3.3	4.4	5.0	6.0	8.2	
RR	1.00	1.03	1.26	1.38	1.28	0.2
CI	...	(0.7, 1.6)	(0.8, 2.0)	(0.9, 2.2)	(0.8, 2.0)	
P	...	0.9	0.3	0.2	0.3	

TABLE 3. Continued

	Quintiles					
	1	2	3	4	5	Trend
ApoE in HDL, mg/dL						
Mean	2.2	3.2	3.9	4.8	7.0	
RR	1.00	1.52	0.97	1.69	2.05	0.002
CI	...	(0.9, 2.4)	(0.6, 1.6)	(1.1, 2.7)	(1.3, 3.2)	
P	...	0.08	0.9	0.03	0.002	
ApoCIII/ApoA-I in HDL, mol/mol						
Mean	0.15	0.2	0.2	0.3	0.4	
RR	1.00	1.08	1.13	1.13	1.57	0.04
CI	...	(0.7, 1.7)	(0.7, 1.8)	(0.7, 1.8)	(1.0, 2.5)	
P	...	0.8	0.6	0.6	0.05	
ApoE/ApoAI, mol/mol						
Mean	0.02	0.03	0.03	0.04	0.06	
RR	1.00	1.53	1.63	1.69	2.07	0.006
CI	...	(0.9, 2.5)	(1.0, 2.7)	(1.04, 2.7)	(1.3, 3.3)	
P	...	0.09	0.05	0.03	0.003	
LDL-cholesterol mg/dL						
Mean	114	128	138	147	164	
RR	1.0	1.19	1.35	0.95	1.73	0.04
CI	...	(0.7, 1.9)	(0.9, 2.2)	(0.6, 1.6)	(1.1, 2.7)	
P	...	0.5	0.2	0.8	0.02	
HDL-cholesterol mg/dL						
Mean	28	34	38	42	54	
RR	1.0	1.02	0.91	0.98	0.79	0.28
CI	...	(0.7, 1.6)	(0.6, 1.4)	(0.6, 1.6)	(0.5, 1.3)	
P	...	0.9	0.7	0.9	0.3	
Triglycerides mg/dL						
Mean	83	112	139	172	247	
RR	1.0	0.93	1.65	1.40	1.58	0.03
CI	...	(0.6, 1.5)	(1.0, 2.6)	(0.9, 2.2)	(1.0, 2.5)	
P	...	0.8	0.03	0.16	0.05	

Relative risks and 95% confidence intervals computed by multiple logistic regression with covariates of age, smoking, hypertension, and left ventricular ejection fraction.

was 3.2 in the full cohort (Figure 3) versus 3.4 in the nondiabetics, and for apoCIII in VLDL+LDL the relative risk was 2.3 versus 1.8, respectively. Thus, the larger number of diabetics among the cases did not account for the predictive association of these lipoprotein measurements with coronary events. The relative risks were slightly attenuated when additional covariates, waist circumference, and use of β-blockers or diuretics were added with diabetes together to the standard model.

The relative risks for VLDL measurements and apoCIII and apoE in VLDL+LDL tended to be higher in the pravastatin group than in the placebo group. For example, the univariate relative risks for the highest compared with the lowest quintile of VLDL-apoB were 1.6 (95% CI 0.4 to 3.0) in the placebo group compared with 2.7 (1.3 to 5.6) in the pravastatin group and for apoCIII in VLDL+LDL 1.5 (0.8 to 2.8) in placebo versus 2.7 (1.3 to 5.7) in the pravastatin group. However, these differences in the relative risks between the placebo and pravastatin groups were not significant by test of interaction ($P=0.09$ for VLDL-apoB, $P=0.25$ for apoCIII in VLDL+LDL).

Discussion

Difficulty in establishing the independent relation between plasma triglycerides and CHD may have been due in part to the lack of specificity of a plasma triglyceride measurement to indicate levels of atherogenic lipoproteins. Triglycerides are carried in lipoproteins, mainly VLDL, which are heterogeneous in size, density, composition, and function.[6,7] We found that the risk of a recurrent coronary event associated with plasma triglycerides could be explained by 2 specific and related measurements, VLDL-apoB (which indicates the concentration of VLDL particles in plasma since each VLDL particle has 1 apoB molecule) and the apoCIII concentration of VLDL+LDL. VLDL-apoB concentration therefore may represent the concentration of potentially atherogenic VLDL particles to which the arterial wall is exposed. Excessive apoCIII may contribute to the atherogenicity of VLDL

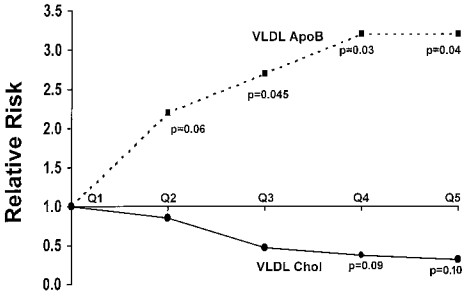

Figure 1. VLDL concentration and risk of recurrent coronary events. VLDL-apoB and VLDL-cholesterol concentrations in quintiles (Q1-Q5) were included together in multiple logistic regression model, along with LDL-cholesterol, HDL-cholesterol, and triglycerides. Other covariates were age, smoking, hypertension, and left ventricular ejection fraction. Median values for quintiles are in Table 3. Probability values compare relative risk for quintiles 2 to 5 compared with quintile 1. Probability values are shown if ≤0.10.

particles, since apoCIII delays the lipolysis of VLDL[22] and inhibits its uptake and clearance from plasma by normal, high-affinity receptors on hepatocytes.[23] Approximately 30% to 70% of VLDL particles contain apoCIII, compared with only a few percent of LDL particles.[24] Inhibition of rapid clearance of VLDL by the liver may be atherogenic if it results in uptake by low-affinity, high-capacity pathways in cells involved in atherosclerosis. Therefore, the relation between the apoCIII concentration in VLDL+LDL and coronary events in the present study gains plausibility from the deleterious metabolic properties of this apolipoprotein.

These findings are consistent with studies that found that apoCIII concentrations in VLDL+LDL were increased in survivors of myocardial infarction[11] and in patients before undergoing bypass surgery[10] compared with control populations. ApoCIII concentrations in VLDL+LDL also were significant markers of progression of coronary atherosclerosis measured by angiography.[12–14] The present study is applicable to the majority of patients in the United States with CHD because it included patients with average LDL-cholesterol concentrations (115 to 174 mg/dL)[18] and excluded those with hypercholestesterolemia (total cholesterol >240 mg/dL), or hypertriglyceridemia (triglycerides >350 mg/dL).

In the present and in previous studies, apoE concentrations in plasma or in VLDL+LDL were associated with CHD,[10,11,15,16] a counterintuitive finding, in view of the necessity for apoE for normal rapid removal of VLDL by the

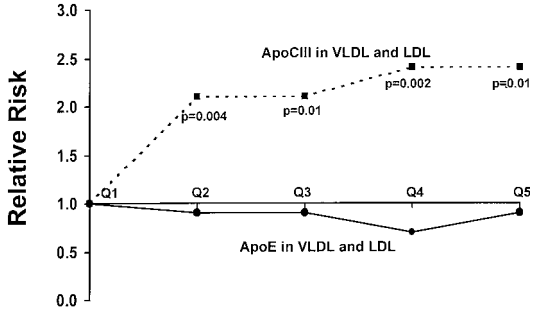

Figure 2. ApoCIII and apoE concentrations in VLDL+LDL and risk of recurrent coronary events. See legend to Figure 1 for methods.

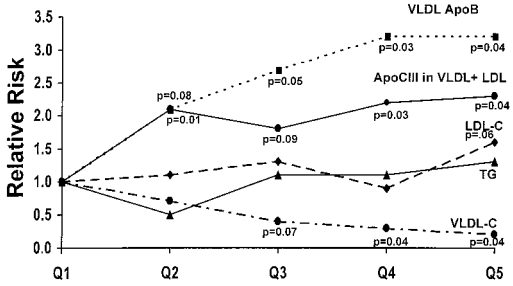

Figure 3. VLDL-apoB concentration and apoCIII concentration in VLDL+LDL and risk of recurrent coronary events. See legend to Figure 1 for methods. For HDL-cholesterol (not shown), relative risks for second to fifth quintiles compared with first quintile were 0.96 to 1.01 (NS).

liver.[9,25] Since most VLDL and LDL particles that contain apoE also contain apoCIII,[24] apoE in VLDL+LDL may be simply a marker for apoCIII and not a direct cause of atherosclerosis. This explanation is supported by multivariate analysis showing that apoCIII in VLDL+LDL was the independent risk factor of the two. Perhaps apoCIII is the dominant of these two metabolically antagonistic apolipoproteins in VLDL and LDL in influencing coronary events in humans.

However, subordinance of apoE to the actions of apoCIII does not explain the finding that HDL-apoE but not HDL-apoCIII was an independent risk factor for coronary events. In a previous study, HDL-apoE independently discriminated patients with coronary bypass surgery from normal control subjects.[10] In the present study, high HDL-apoE concentration was correlated with low HDL-cholesterol and high triglycerides, VLDL-apoB, and VLDL/LDL apoCIII concentrations. Recently, this lipoprotein pattern was produced by overexpression of human apoE in transgenic mice.[26] The mechanism was stimulation by apoE of VLDL production by the liver and inhibition by apoE of VLDL lipolysis by lipoprotein lipase. Thus, the association between HDL-apoE and an atherogenic lipid profile and risk of coronary events could be directly related to the actions of apoE itself.

The VLDL cholesterol concentration, a significant positive predictor of coronary events in univariate analysis, became an inverse predictor when VLDL-apoB was included in the multivariate model. Consistent with this unexpected finding, the cholesterol enrichment of VLDL particles was inversely related to risk of coronary events. Cholesterol-rich VLDL particles are taken up rapidly by LDL receptors on cultured fibroblasts and macrophages.[27] Although this may appear to be an atherogenic characteristic of VLDL, rapid uptake of cholesterol-rich VLDL by LDL receptors in the liver is the normal route for VLDL clearance, which could protect against their entry into the arterial intima.

The primary analysis of this study included the total cohort of patients who had a recurrent event, whether treated with pravastatin or placebo. The relative risks associated with VLDL-apo B, and apoCIII in VLDL+LDL appeared to be stronger in the pravastatin group than in the placebo group. However, this apparent difference was not statistically significant and therefore may have been due to chance. We recently reported that pravastatin reduced the concentrations

of these apolipoprotein measurements (Sacks FM, et al, 1999 AHA Scientific Sessions). Nonetheless, statin therapy, by diminishing the influence of LDL on CHD, could "unmask" the influence of atherogenic VLDL particles or have differential effects on VLDL particle types with distinct atherogenic potential.

In summary, we found that the VLDL-apoB concentration, the apoCIII concentration of VLDL+LDL, and apoE in HDL are independent predictors of recurrent coronary events and explain the weaker relation between plasma triglycerides and coronary events. The results are consistent with the known metabolic properties of apoCIII and VLDL particles, which link them to atherosclerosis, and with newly recognized properties of apoE and suggest that plasma triglyceride level is an imperfect marker for these specific lipoprotein particle measurements. The apolipoprotein measurements are not technically difficult. These prospective findings need to be explored in other populations, and if confirmed could be applied clinically.

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