

LDL Containing Apolipoprotein CIII Is an Independent Risk Factor for Coronary Events in Diabetic Patients

Sung-Joon Lee, Hannia Campos, Lemuel A. Moye, Frank M. Sacks

Objective—Triglyceride-rich lipoproteins that contain apolipoprotein CIII (apoCIII) are prominent in diabetic dyslipidemia. We hypothesized that these lipoproteins increase coronary disease risk in diabetic patients beyond that caused by standard lipid risk factors.

Methods and Results—Diabetic patients with previous myocardial infarction were followed for 5 years, and 121 who had a recurrent coronary event were matched to 121 who did not. VLDL and LDL that contained or did not contain apoCIII (CIII+ or CIII−) were prepared by immunoaffinity chromatography and ultracentrifugation. IDL was included in the LDL fraction. LDL CIII+, rich in cholesterol and triglyceride, was the strongest predictor of coronary events (relative risk [RR] 6.6, $P < 0.0001$, for 4th versus 1st quartile). LDL CIII+ comprised 10% of total LDL. The main type of LDL, LDL CIII−, was less strongly predictive (RR 2.2, $P = 0.07$). The increased risk associated with LDL CIII+ was unaffected by adjustment for plasma lipids, apoB, non-HDL cholesterol, or the other VLDL and LDL types. For VLDL CIII+, RR 0.5, $P = 0.07$; for VLDL CIII−, RR 2.3, $P = 0.046$. The presence of apolipoprotein E with CIII on VLDL and LDL did not affect risk.

Conclusions—LDL with apoCIII strongly predicts coronary events in diabetic patients independently of other lipids and may be an atherogenic remnant of triglyceride-rich VLDL metabolism. (*Arterioscler Thromb Vasc Biol.* 2003;23:853-858.)

apolipoprotein CIII ■ lipoproteins ■ coronary heart disease ■ apolipoprotein E ■ apolipoprotein B

Patients with non-insulin-dependent diabetes (NIDDM) have 2 to 3 times higher the risk of coronary heart disease (CHD) than nondiabetic patients.¹⁻⁴ Plasma cholesterol,^{1,5} LDL cholesterol,^{1,5} and HDL cholesterol (HDL-C)^{1,5} are strong risk factors for CHD in NIDDM. Diabetic patients have higher plasma triglyceride concentrations than nondiabetic patients. It is not entirely clear whether the high TG concentration contributes independently to CHD in diabetes. In the Paris Prospective Study, TG concentration was an independent predictor in people with impaired glucose tolerance or diabetes even after adjustment for HDL-C and other risk factors.⁶ However, in the much larger United Kingdom Prospective Diabetes Study (UKPDS), high TG was significant only in univariate but not in multivariate analysis that included HDL-C.⁵ Lack of independence of triglyceride as a risk factor in UKPDS was not caused by greater methodological or biological variability for triglycerides compared with the other lipid risk factors.⁵

The metabolism of triglyceride-rich lipoproteins, chylomicrons, and some types of VLDL and LDL is abnormal in NIDDM. The production of triglyceride and VLDL by the

liver is elevated,⁷⁻⁹ and the activity of lipoprotein lipase, which metabolizes triglyceride in VLDL, is decreased.¹⁰ It is possible that the plasma triglyceride concentration does not capture the full adverse effect on CHD of abnormal metabolism of triglyceride-rich lipoproteins. The same concern may apply to other lipid measurements that include triglyceride-rich lipoproteins, such as total apolipoprotein B (apoB) or non-HDL cholesterol.

ApoCIII is a small protein on the surface of apoB lipoproteins strongly affecting their metabolism. Alaupovic and colleagues¹¹⁻¹³ recognized that VLDL and LDL (apoB lipoproteins) contain subpopulations that have apoCIII and hypothesized that classification of apoB lipoproteins on the basis of apoCIII content would yield metabolically diverse types with distinct relationships to atherosclerosis. They found that apoCIII was increased in apoB lipoproteins in patients with diabetes.¹² ApoCIII is an inhibitor of the activity of lipoprotein lipase,¹⁴ which metabolizes triglyceride in VLDL and facilitates their clearance from plasma. ApoCIII also obstructs the clearance from plasma of VLDL and LDL by interfering with their interaction with hepatic lipoprotein

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From the Department of Nutrition, Harvard School of Public Health (S.J.L., H.C., F.M.S.) and the Department of Medicine, Harvard Medical School and Brigham and Women's Hospital (F.M.S.), Boston, Mass, and University of Texas School of Public Health (L.A.M.), Houston, Tex. Dr Lee is presently at the Stanford School of Medicine, Calif.

Correspondence to Frank M. Sacks, MD, Nutrition Department, Harvard School of Public Health, 665 Huntington Ave, Boston, MA 02115. E-mail fsacks@hsph.harvard.edu

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receptors.^{15,16} The plasma concentration of apoCIII in VLDL and LDL is associated with CHD,^{17–20} including patients with NIDDM.¹⁸ Thus, we hypothesized that VLDL or LDL that contains apoCIII (apoCIII+) is a predictor of subsequent CHD events in NIDDM.

We additionally hypothesized that apoE would lessen the adverse effects of apoCIII on VLDL or LDL on CHD, because apoE functions as a ligand for cell-surface receptors that take up VLDL and LDL from plasma.²¹ To determine directly whether the presence of apoE affects CHD, it is necessary to separate and study VLDL and LDL that have just apoCIII and those that have both apoCIII and apoE. In this study, we investigated the association of VLDL and LDL containing apoCIII, both apoCIII and apoE, or neither with recurrent coronary events in 242 patients with diabetes.

Methods

Subjects and Blood Samples

We conducted a prospective, nested case-control study in the Cholesterol and Recurrent Events (CARE) trial, a trial of pravastatin, an HMG-CoA reductase inhibitor, in 4159 patients who had experienced myocardial infarction (MI).²² Fasting cholesterol level <240 mg/dL, LDL cholesterol 115 to 174 mg/dL, triglyceride <350 mg/dL, and glucose <220 mg/dL were inclusion criteria. Institutional review boards of the centers approved the study, all patients gave informed consent, and the procedures followed were in accordance with institutional guidelines.

There were 586 diabetic patients in the CARE trial. A total of 193 of them had at least one of the following recurrent coronary events during 5 years of follow-up: MI, coronary artery bypass grafting (CABG), or percutaneous transluminal coronary angioplasty (PTCA). Of these, 122 could be matched to diabetic patients who did not have a recurrent coronary event or stroke during follow-up using the matching criteria, age within 15 years, and sex. The baseline characteristics of the 122 diabetic patients with matches were very similar to those of the 71 without matches, eg, age 61 versus 62 years, men 83% versus 77%, body mass index 29 versus 30, smokers 16% versus 10%, and hypertensives 52% versus 52%. Baseline mean triglycerides, LDL-C, and HDL-C were the same in matched and unmatched cases. Of the 122 matched diabetic patients, 1 had insufficient plasma for analysis. Thus the final study group included 121 diabetic patients with recurrent events and their matched controls.

Laboratory Methods

Blood was collected in tubes containing EDTA after the patients had fasted for longer than 8 hours. Blood samples were sent by overnight delivery to the core laboratory in St Louis, Missouri, where the baseline laboratory measurements were performed on fresh plasma. Plasma aliquots were placed in 1-mL vials and stored at -80°C . One vial containing 1 mL of frozen plasma was shipped to Dr Sacks's laboratory at the Harvard School of Public Health for analysis of lipoprotein types. Analyses were conducted by Dr Lee on 3 case-control paired samples at a time. None of the investigators at the laboratory was aware of the group identification.

Immunoaffinity chromatography was conducted with affinity-purified anti-apoCIII and anti-apoE on Sepahacryl S1000 resin, as previously described and validated.^{23,24} Plasma (1 mL) was thawed and incubated overnight with anti-apoE immunoaffinity resin. The unbound lipoproteins that did not contain apoE (E-) were collected and incubated overnight on an anti-apoCIII column to isolate those with (E-CIII+) and without apoCIII (E-CIII-). The bound lipoproteins that contained apoE (E+) were eluted with 3 mol/L NaSCN and desalted by gel filtration. Because >95% of E+ particles also had apoCIII,^{23–25} they were not separated additionally and were denoted as E+CIII+ in the present study. The E+CIII- particles, especially in the LDL density range, were not detectable over 90% of diabetic

patients. In the nomenclature of Alaupovic,¹³ these particle types would be labeled Lp-B:C (for E-CIII+), Lp-B:C:E (for E+CIII+), and Lp-B (for E-CIII-). The particle type identified by Alaupovic that has apoD and apoA-II in addition to apoB, E, and CIII would be included in the E+CIII+ particles in our study.

VLDL ($d < 1.006$) and LDL ($1.006 < d < 1.050$) were isolated from 3 immunofractions of plasma (E+CIII+, E-CIII+, and E-CIII-) by very-fast ultracentrifugation following the methods of Pietzsch et al²⁶ and Leonhardt et al.²⁷ A Beckman Optima TLX ultracentrifuge, a Beckman TLA 120.2 fixed-angle rotor, and thick-wall polycarbonate tubes (No. 343778, Beckman) were used. To prepare VLDL, we transferred 600 μL of the concentrated immunofractions (E+CIII+, E-CIII+, and E-CIII-) to ultracentrifuge tubes. The samples were overlaid with 400 μL of $d = 1.006$ kg/L and submitted to ultracentrifugation (1 hour 17 minutes, 15°C , 625 000g). The VLDL in the top 400 μL of each tube was carefully harvested by aspiration. To prepare LDL, we mixed 81.5 μL of 40% saturated KBr solution with 18.5 μL of double-distilled water and added the 100 μL of KBr solution to the lower fraction to adjust the density to 1.050. The final volume was made up to 1 mL with $d = 1.050$. The solute density was adjusted and verified by an optical densitometer (Bausch & Lomb). Centrifugation was performed (2 hours 30 minutes, 15°C , 625 000g), and 400 μL of the supernatant, containing LDL, was collected. The run time and speeds were standardized by comparison with the classic Lindgren method using a SW41 swinging bucket rotor (Beckman).²⁸ As a result, the following 6 lipoprotein types were isolated from the plasma of each patient: VLDL E+CIII+, VLDL E-CIII+, VLDL E-CIII-, LDL E+CIII+, LDL E-CIII+, and LDL E-CIII-. In each of these lipoprotein types, apoB was measured by ELISA and cholesterol and triglyceride by enzymatic methods (Cobas Mira Plus, Roche Diagnostics).

We used $d < 1.050$ rather than $d < 1.063$ for LDL to isolate relatively pure LDL, minimizing contamination with lipoprotein (a) and large HDL particles. We found that recovery of LDL was virtually complete. Using non-HDL cholesterol as determined by the standard precipitation method (total cholesterol - HDL cholesterol) as the reference value, recovery of cholesterol in the sum of VLDL, IDL, and LDL was 99% and recovery of triglyceride was 98%. Finally, the bottom fraction after the LDL spin contained <5% of total apoB, indicating that we collected almost all LDL particles in the density <1.050.

Unpublished data from a smaller group of diabetic patients from the CARE study showed that the distribution of E-CIII+ in diabetic patients is 65% in LDL, 9% in IDL, and 26% in VLDL. For E+CIII+ particles, the distribution is 61% in LDL, 7% in IDL, and 32% in VLDL; and for E-CIII- particles, the distribution is 94% in LDL, 3% in IDL, and 3% in VLDL. Thus, 88% to 97% of the fraction-labeled LDL in the present study is LDL.

Statistical Analysis

Statistical analyses were performed at the Harvard School of Public Health using the Statistical Analysis Systems software, version 8.0 (SAS Institute, Inc). The differences between cases and controls were analyzed using a paired *t* test. The associations of lipoprotein types with recurrent coronary events were computed using logistic regression analysis. The distribution of measurements of lipoprotein types of the controls was divided into quartiles, and the cases and controls in each quartile were counted. The relative risks (RRs) and 95% confidence intervals (CIs) of the 2nd, 3rd, and 4th quartiles were then computed using the 1st quartile as a reference group. Univariate regression models of RR on VLDL and LDL types were performed without adjustment for risk factors and other covariates. Each VLDL and LDL type was evaluated in multivariate analysis with covariates of age and sex (the matching factors), treatment group (placebo or pravastatin), waist circumference, exercise, history of angina or CABG, fasting glucose, use of oral hypoglycemic medication, and plasma triglyceride, LDL cholesterol, and HDL cholesterol to determine whether the VLDL and LDL types predicted events independently of the standard lipid risk factors, other risk factors, and interventions. Plasma total apoB or non-HDL cholesterol was substituted for LDL cholesterol in additional models. The

TABLE 1. Baseline Characteristics of Case and Control Groups

	Controls	Cases	P Value
n	121	121	
Triglyceride, mg/dL	165±69	164±66	0.88
Cholesterol, mg/dL			
Total	206±17	207±16	0.74
LDL	135±14	136±14	0.66
HDL	38±10	38±9	0.95
non-HDL	168±18	169±16	0.77
Glucose, mg/dL	143±49	149±52	0.35
Age, y	61±8	61±9	0.72
Male sex	83	83	1
White race	86	87	0.71
Alcohol, drinks/wk			0.30
None	76	73	
1–4	17	17	
5–10	5	3	
≥11	2	10	
Current smoker	11	16	0.26
Systolic blood pressure	131±20	134±19	0.19
Diastolic blood pressure	77±10	78±11	0.46
Diabetes	100	100	—
Hypertension	49	63	0.61
Angina	21	33	0.04
CABG	35	19	0.006
PTCA	27	26	0.77
LVEF	52±13	51±12	0.65
Body mass index, kg/m ²	29±5	29±5	0.41
Waist circumference, cm	99±12	102±16	0.06
Medication use			
Oral hypoglycemic	40	50	0.09
β-blockers	35	43	0.19
ACE inhibitor	21	24	0.65
Diuretics	21	23	0.76
Insulin	13	20	0.17
Estrogen	2	2	0.65

Values are percent of patients of population except as indicated. Plus-minus values are mean±SD.

CVD indicates cardiovascular diseases; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme.

influence of adjusting for the standard lipid risk factors was determined by leaving them out of the model. LDL cholesterol was the standard clinical measurement.²⁹ *P*<0.05 (2-sided) was regarded as significant.

Results

Characteristics of Cases and Controls

The concentrations of standard lipid risk factors, triglyceride, total cholesterol, LDL cholesterol, HDL cholesterol, non-HDL cholesterol, and glucose were similar between groups (Table 1). Baseline characteristics of this sample of the diabetic patients were similar to those of the entire group of 586 diabetic patients.³⁰ Among the controls, 50% received

TABLE 2. VLDL and LDL Containing apoCIII or apoE in Cases and Controls

	Controls (n=121)	Cases (n=121)	P Value
VLDL E+CIII+			
apoB	1.9±1.2	1.8±1.5	0.9
Cholesterol	11.4±7.1	9.9±5.8	0.18
Triglyceride	66±44	60±40	0.20
Cholesterol/apoB	8500±8300	7700±5600	0.9
Triglyceride/apoB	22000±23000	21000±17000	0.6
VLDL E–CIII+			
apoB	0.89±0.80	0.85±0.84	0.9
Cholesterol	4.1±3.5	3.6±2.8	0.5
Triglyceride	24±21	21±16	0.2
Cholesterol/apoB	6500±6300	6000±4800	0.4
Triglyceride/apoB	17000±17000	16000±12000	0.4
VLDL E–CIII–			
apoB	3.0±1.6	3.5±2.2	0.0009
Cholesterol	4.0±4.8	6.6±3.2	0.2
Triglyceride	20±9	22±11	0.07
Cholesterol/apoB	1900±4200	2700±2200	0.2
Triglyceride/apoB	4100±3700	3900±3300	0.08
LDL E+CIII+			
apoB	4.7±1.7	5.4±2.4	0.0001
Cholesterol	19±6	20±7	0.005
Triglyceride	11±5	12±6	0.08
Cholesterol/apoB	5800±5100	5400±4200	0.10
Triglyceride/apoB	1400±1900	1400±1500	0.20
LDL E–CIII+			
apoB	4.6±2.4	5.6±3.2	0.0002
Cholesterol	18±10	20±11	0.02
Triglyceride	6.8±3.5	7.9±3.9	0.01
Cholesterol/apoB	5400±5700	5100±5200	0.08
Triglyceride/apoB	930±910	900±770	0.13
LDL E–CIII–			
apoB	83±25	86±27	0.003
Cholesterol	112±29	112±29	0.13
Triglyceride	18±5	19±8	0.02
Cholesterol/apoB	1940±1700	1860±1500	0.15
Triglyceride/apoB	134±135	143±178	0.7

apoB, cholesterol, and triglyceride values are mg/dL. Cholesterol/apoB and triglyceride/apoB are molar ratios (molecules of lipid per VLDL or LDL particle). LDL includes IDL particles. In the nomenclature of Alaupovic,¹³ E+CIII+ is Lp-B:C:E, E–CIII+ is Lp-B:C, and E–CIII– is Lp-B.

pravastatin treatment compared with 42% of the cases, a nonsignificant difference (*P*=0.22).

VLDL and LDL Types in Cases and Controls

The apoB concentrations of VLDL E–CIII–, LDL E–CIII–, LDL E–CIII+, and LDL E+CIII+ were significantly higher in cases than controls (Table 2). In each instance, the cholesterol and triglyceride concentrations of these lipoprotein types were also higher in cases, although

TABLE 3. Relative Risks for VLDL and LDL Types

		Quartiles			
		1	2	3	4
VLDL CIII+	Mean	0.8	1.4	2.3	3.9
Unadjusted	RR (CI)	1	0.5 (0.3 to 1.05)	0.7 (0.4 to 1.4)	0.6 (0.3 to 1.1)
	<i>P</i>		0.07	0.3	0.10
Adjusted	RR (CI)	1	0.5 (0.2 to 1.0)	0.6 (0.3 to 1.3)	0.5 (0.2 to 1.1)
	<i>P</i>		0.06	0.2	0.07
VLDL CIII-	Mean	0.9	1.9	2.4	3.8
Unadjusted	RR (CI)	1	0.7 (0.3 to 1.5)	0.6 (0.3 to 1.2)	1.9 (0.9 to 3.7)
	<i>P</i>		0.4	0.2	0.07
Adjusted	RR (CI)	1	1.0 (0.4 to 2.3)	0.5 (0.2 to 1.3)	2.3 (1.0 to 5.3)
	<i>P</i>		1.0	0.2	0.046
LDL CIII+	Mean	4.5	5.9	7.2	10.4
Unadjusted	RR (CI)	1	2.5 (1.0 to 6.1)	1.8 (0.7 to 4.6)	6.1 (2.6 to 14)
	<i>P</i>		0.04	0.2	<0.0001
Adjusted	RR (CI)	1	3.0 (1.2 to 7.6)	1.7 (0.6 to 4.7)	6.6 (2.6 to 17)
	<i>P</i>		0.02	0.3	<0.0001
LDL CIII-	Mean	42	55	65	86
Unadjusted	RR (CI)	1	0.8 (0.4 to 1.8)	1.3 (0.6 to 2.6)	1.7 (0.8 to 3.4)
	<i>P</i>		0.6	0.5	0.15
Adjusted	RR (CI)	1	1.0 (0.4 to 2.2)	1.6 (0.7 to 3.7)	2.2 (0.9 to 5.0)
	<i>P</i>		0.9	0.2	0.07

Mean values are apolipoprotein B concentrations (mg/dL).

Unadjusted indicates no covariates included; Adjusted, Baseline triglyceride, LDL cholesterol, HDL cholesterol, age, sex, exercise, waist circumference, CABG, angina, glucose, oral hypoglycemic use, and treatment group (placebo or pravastatin) were included.

LDL includes the IDL fraction. In the nomenclature of Alaupovic,¹³ CIII+ is Lp-B:C and CIII- is Lp-B.

less so in magnitude or level of statistical significance than apoB. Thus, apoB was used as the measure of concentration of VLDL and LDL types for subsequent analyses. In contrast, cases and controls did not significantly differ in mean concentrations of VLDL E-CIII+ and VLDL E+CIII+. Because we found that the relationship with coronary events of the 2 apoCIII containing VLDL and LDL particle types, E-CIII+ and E+CIII+, was nearly identical, we combined these particle types in subsequent primary analyses. Thus, the particle types are expressed as CIII+ or CIII-.

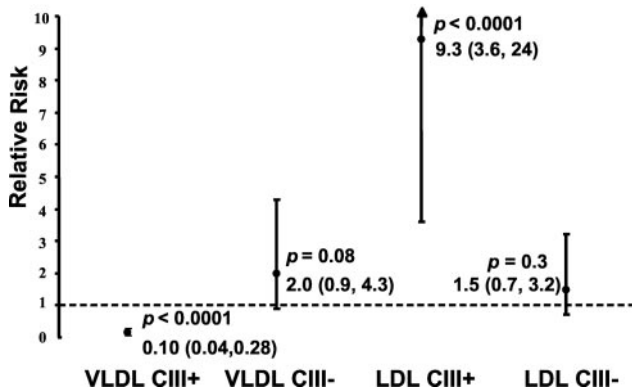
Relative Risk Predicted by VLDL and LDL Types

LDL CIII+ was the strongest predictor of recurrent coronary events among the VLDL and LDL particle types, RR 6.6 (95% CI, 2.6 to 17), $P<0.0001$ for 4th versus 1st quartile, in multivariate analysis, adjusted for treatment group (pravastatin or placebo), risk factors, LDL cholesterol, HDL cholesterol, and triglycerides (Table 3). RR for high LDL CIII+ was similarly increased in this multivariate model that substituted plasma total apoB or non-HDL cholesterol for LDL cholesterol, 5.2 ($P=0.0005$) and 6.5 ($P<0.0001$), respectively. Leaving out the lipid risk factors from the multivariate analysis had little effect on the RR for LDL CIII+ (5.6, $P=0.0001$). Univariate analysis (no adjustment for treatment group or risk factors) yielded similarly increased risk for LDL

CIII+, RR 6.1 (95% CI, 2.6 to 14), $P<0.0001$ (Table 3). A high LDL CIII+ concentration was associated with increased risk in patients with plasma total apoB below or above the median concentration, 98 mg/dL, RR 5.9 ($P=0.047$) and RR 8.3 ($P=0.004$), respectively. LDL CIII+ particles comprised 10% of the total LDL and were highly enriched in cholesterol and triglycerides compared with LDL CIII- particles (Table 2), and their concentration was mildly although significantly correlated with plasma triglycerides, $r=0.24$ ($P<0.001$). The RR in multivariate analysis for the subdivided LDL CIII+, that is LDL E-CIII+ and LDL E+CIII+, were 3.0 (95% CI, 1.2 to 7.4) and 2.1 (95% CI, 1.0 to 4.6), respectively.

The major LDL particle type, LDL CIII-, comprising 90% of LDL particles, was also associated with increased risk of coronary events in multivariate analysis (RR 2.2; 95% CI, 0.9 to 5.0; $P=0.07$), although less strongly than LDL CIII+ (Table 3). The RR was 1.96 ($P=0.09$) in a multivariate model that included risk factors but not lipids and 1.7 ($P=0.15$) in univariate analysis. LDL CIII- had no correlation with triglycerides, $r=-0.03$.

The VLDL types had a complex relationship to coronary events. VLDL CIII- was associated with increased RR (2.3; 95% CI, 1.0 to 5.3; $P=0.046$) in multivariate analysis (Table 3). Substituting plasma total apoB or non-HDL cholesterol for LDL cholesterol in the model had little effect on risk, RR 1.9 ($P=0.12$) and 2.3 ($P=0.047$), respectively, nor did



Multivariate analysis of VLDL and LDL types as predictors of recurrent coronary events in diabetes. The apoB concentrations of VLDL and LDL particle types were included together. The LDL fraction includes IDL.

leaving out the lipid risk factors (RR 1.96, $P=0.08$). In contrast, VLDL CIII+ was associated with decreased RR, although not statistically significantly (RR 0.5; 95% CI, 0.2 to 1.1; $P=0.07$) in multivariate analysis (Table 3). Again, leaving out the lipid risk factors had little effect on RR (0.5, $P=0.06$). VLDL CIII+ was much enriched with triglyceride and cholesterol compared with VLDL without apoCIII (Table 2). The RRs in multivariate analysis for the subdivided VLDL CIII+, that is VLDL E-CIII+ and VLDL E+CIII+, were 0.7 (95% CI, 0.3 to 1.7) and 0.9 (95% CI, 0.4 to 1.9), respectively.

In an additional multivariate model, LDL CIII+, LDL CIII-, VLDL CIII+, and VLDL CIII- were included together to examine the relative predictive strength of these 4 particle types. The RR for LDL CIII+ remained strong and highly significant (9.3, $P<0.0001$) (Figure). The RRs for the other lipoprotein types were similar to those in previous models (compare the Figure and Table 3), except that the reduced RR for high concentrations of VLDL CIII+ became highly significant (RR 0.10, $P<0.0001$). Finally, the RRs were studied in the individual treatment groups, and no difference was found in the effect of the lipoprotein particle types on risk between pravastatin and placebo groups.

Discussion

The principal finding is that the plasma concentration of LDL particles that have apoCIII strongly predicted recurrent coronary events in diabetic patients who had had a myocardial infarction. This relationship between LDL CIII+ and coronary events was independent of the standard lipid risk factors, plasma total apoB, non-HDL cholesterol, other baseline characteristics of the patients, and pravastatin treatment, thus demonstrating considerable robustness. The LDL CIII+ particles seem to be quite potent, because a rather small increase in concentration of only 6 mg/dL, comparing the medians of the 1st versus 4th quartiles, corresponded to a 6-fold increased risk of a coronary event.

The LDL CIII+ particles comprise 10% of the plasma concentration of all LDL particles and are differentiated from the predominant LDL particle type that does not have apoCIII by their large size,²⁴ high cholesterol and triglyceride con-

tent,^{23,24} and positive correlation with plasma triglyceride. This result is in line with the recent finding that large LDL particles are predictors of recurrent coronary events.³¹ Their precursor is likely to be VLDL CIII+, because these VLDL and LDL types have similarly high cholesterol content and differ only in reduced triglyceride content in LDL CIII+. These findings suggest that the LDL CIII+ particles result from partial lipolysis of triglyceride-rich VLDL particles and may be the remnant lipoproteins that have long been proposed to be atherogenic.^{13,32,33} The LDL CIII+ in the present study includes IDL particles, and apoCIII may also contribute to the relationship between IDL and progression of coronary and carotid atherosclerosis.^{34,35}

In contrast to the highly increased risk associated with LDL CIII+, VLDL CIII+ was associated with moderately reduced RR of approximately 50%, although not reaching statistical significance. VLDL CIII+ are huge particles²⁴ that we speculate are unable to effectively pass through arterial endothelial cells into the intima to form plaque, and they become atherogenic only after shrinkage by triglyceride lipolysis. In contrast to VLDL CIII+, VLDL apoCIII- have much lower triglyceride and cholesterol contents and are smaller in size.²⁴ VLDL CIII- was associated with moderately increased risk, approximately 2-fold, similar to LDL CIII-.

The presence of apoE on LDL CIII+ and VLDL CIII+ particles had little apparent relationship to coronary events. LDL E-CIII+ and LDL E+CIII+ were both highly significantly and similarly increased in cases. This is consistent with our previous report in the CARE population that apoE concentration in VLDL and LDL was not a predictor of coronary events in multivariate analysis that included the apoCIII concentration in VLDL and LDL.¹⁷ ApoE should enhance VLDL clearance from plasma by the liver²¹ and thus be beneficial. We recently reported in healthy persons that VLDL particles that had apoE were actually catabolized more slowly than those that did not have apoE, and we attributed this apparently paradoxical result to a much higher content of inhibitory apoCIII in VLDL apoE+ than VLDL apoE-.³⁶ Thus, apoCIII may play the dominant adverse role in VLDL and LDL metabolism and relationship with CHD.

These results refine those of previous reports that linked apoCIII in VLDL and LDL together with CHD.¹⁷⁻²⁰ Measurements of the combined VLDL and LDL types include a diverse group of lipoprotein types that show qualitative and quantitative differences as regards CHD. Thus, enhanced specificity in measurement may improve the quantification of risk and targeting of therapy. In conclusion, the present study points toward apoCIII containing LDL particles as a potential target for diet and pharmacological therapy to reduce the high rate of coronary events in diabetic patients. This conclusion could be extended to nondiabetic persons, because they also have LDL apoCIII+,²³⁻²⁵ Thus, this lipoprotein type as a CHD risk factor needs to be investigated in the general population.

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