ELSEVIER

Atherosclerosis 167 (2003) 293–302

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Hypertriglyceridemia but not diabetes status is associated with VLDL containing apolipoprotein CIII in patients with coronary heart disease

Sung-Joon Lee [a,1], Lemuel A. Moye [b], Hannia Campos [a], Gordon H. Williams [c], Frank M. Sacks [a,d,*]

[a] *Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, USA*
[b] *University of Texas School of Public Health, Houston, TX 77030, USA*
[c] *Endocrine-Hypertension Division, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115, USA*
[d] *Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115, USA*

Received 5 April 2002; received in revised form 6 October 2002; accepted 20 November 2002

Abstract

High apoCIII concentration in apoB lipoproteins is a prominent component of atherogenic dyslipidemia, and explains the risk of coronary heart disease (CHD) associated with high triglyceride (TG). We hypothesized that diabetic people have atherogenic dyslipidemia with apoCIII in excess of that accounted for by their high TG levels. We selected 30 diabetic and 30 nondiabetic persons, 15 of each with fasting TG < 160 mg/dl and 15 with TG ≥ 200 mg/dl. Using immunoaffinity chromatography and ultracentrifugation, we prepared large and small VLDL, IDL and LDL with or without apoCIII or apoE. The groups with TG $\geq$ 200 mg/dl, regardless of diabetes status, had higher concentrations of large and small VLDL particles with apoCIII and higher apoCIII concentrations than the groups with fasting TG < 160 mg/dl. The diabetes groups did not have higher concentrations of these lipoproteins than the nondiabetes groups within the same fasting TG criteria. In conclusion, high concentrations of apoCIII-containing VLDL are associated with hypertriglyceridemia, which may play a critical role in identifying the high risk of CHD in hypertriglyceridemic patients whether diabetic or nondiabetic. Diabetes status per se does not appear to be associated with high concentrations of apoCIII-containing TG-rich lipoprotein particles, if the plasma TG levels are similar.

Keywords: Coronary heart disease; Apolipoprotein B, E, and CIII; Lipoproteins; Diabetes

1. Introduction

Diabetic patients have higher plasma triglyceride (TG) concentrations than nondiabetics, and considerably higher rates of coronary heart disease (CHD). 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 [1]. However, in the much larger UKPDS, high TG was significant only in univariate but not in multivariate analysis that included HDL-C [2]. VLDL and IDL concentrations are high in diabetic patients [3] due to a high VLDL production rate [4–6] and a slow clearance of VLDL [7,8]. The plasma concentration of apolipoprotein CIII (apoCIII) is elevated in diabetic patients [9,10], and is positively correlated with TG levels [11]. ApoCIII delays metabolism of TG-rich particles by inhibiting lipoprotein lipase activity [12–14] and apoE-dependent receptor binding [15–18], which increases the probability of deposition of cholesterol of lipoprotein particles in the vessel wall. It is possible that the plasma TG concentration does not capture the full adverse effect on CHD of abnormal TG and VLDL metabolism in diabetic patients.

* Corresponding author.
E-mail address: fsacks@hsph.harvard.edu (F.M. Sacks).
[1] Present address: Department of Medicine, Research Institute, Palo Alto Medical Foundation, Stanford University School of Medicine, Palo Alto, CA 94301, USA.

0021-9150/03/$ - see front matter
doi:10.1016/S0021-9150(03)00007-8

The plasma level of apoCIII in apoB lipoproteins is an independent risk factor for CHD [19,20], and for worsening of coronary atherosclerosis [21], and in one crossectional study, it was associated with atherosclerotic vascular disease in NIDDM [22]. In multivariate analysis, apoCIII in VLDL+LDL was a much stronger risk factor than TGs for coronary events, and accounted for the univariate association between TGs and coronary events [19]. It is possible that apoCIII may be important especially in diabetes, since the apoCIII gene has an insulin response element [23,24] which decreases apoCIII production. Since the plasma levels of apoCIII in apoB lipoproteins are strongly correlated with fasting TG levels [11,19,25], it has not been clearly shown whether patients with diabetes have higher plasma levels of apoCIII than patients without diabetes, if their TG levels are similar. A higher apoCIII concentration could help explain the high incidence of CHD in diabetic people. Therefore, we hypothesized that plasma concentrations of apoB lipoproteins with apoCIII, (apoCIII+) would be higher in diabetic than nondiabetic patients with CHD, and help explain the higher rate of CHD in diabetics. To test this hypothesis, we divided both diabetic and nondiabetic patients into two groups based on their fasting TG levels and compared the particle concentrations of apoCIII-containing apoB lipoproteins between diabetic and nondiabetic groups with similar TG levels.

In addition to apoCIII, we considered the role of apoE in diabetic dyslipidemia. Metabolically, apoE contributes to clear VLDL and LDL particle from plasma, functioning as a primarily ligand for both LDL receptor [26] and LDL receptor-related protein [27]. Thus, we would expect that the apoE in VLDL would be a protective influence against CHD. Suprisingly, apoE level in apoB lipoproteins is high in CHD [19,20,28–30]. Since most of apoE coexists with apoCIII in humans [31,32], apoE on apoB particles may simply be a marker for the presence of apoCIII. Therefore, we isolated apoB containing lipoproteins based on the presence of both apoE and CIII to define more precisely the role of each apolipoprotein in diabetic dyslipidemia.

2. Materials and methods

2.1. Subjects and study design

We selected 60 patients from the population in the Cholesterol and Recurrent Events (CARE) trial. The CARE trial was a randomized, double-blinded, placebo-controlled trial of pravastatin in 4159 patients who had myocardial infarction (MI) 3–20 months prior to enrollment in which 14% ($N = 586$) of patients had diabetes at baseline [33]. The study design and the characteristics of diabetic patients were described previously [34,35]. In brief, inclusion criteria were cholesterol < 240 mg/dl, LDL cholesterol 115–174 mg/dl, fasting TG levels < 350 mg/dl, and fasting glucose levels < 220 mg/dl. Institutional Review Boards of the centers approved the study, all patients gave informed consent, and the procedures were in accordance with institutional guidelines. For the present study, the diabetic patients were identified by a self-reported history of diabetes and a fasting blood glucose ≥ 126 mg/dl. Nondiabetic patients were identified by an absence of history of diabetes and a fasting glucose < 110 mg/dl. The diabetic and nondiabetic groups were divided into two groups on the basis of their fasting TG either ≥ 200 mg/dl (HTG) or < 160 mg/dl (NTG). Thus, four groups were established; diabetes with hypertriglyceridemia (DM-HTG), diabetes with normal triglycerides (DM-NTG), nondiabetic with hypertriglyceridemia (nonDM-HTG), and nondiabetic with normal triglycerides (nonDM-NTG). We placed a gap between the criteria for normal and high TG levels to amplify the distinction between the groups. Age (5-year interval) and sex were matching factors among the four groups. Patients who were taking sulfonylurea or acarbose were eligible for inclusion, whereas those taking metformin, insulin or medications affecting lipid metabolism including estrogen, progesterone, beta-blockers and diuretics were excluded. Since plasma from the baseline period of the trial was used in this study, no patients were taking lipid-lowering medications at the time of blood sampling.

2.2. Preparation of blood samples

Fasting blood was sent by overnight delivery in cold containers to the core laboratory in St. Louis, MO. Plasma was prepared by centrifugation at 4 °C; 1-ml aliquots were stored in polypropylene tubes at −80 °C. Vials containing frozen plasma were shipped on dry ice by overnight delivery from the core laboratory to the Harvard School of Public Health in Boston. Analyses were conducted with four samples at a time, including one subject from each of the four groups. None of the personnel at the laboratories were aware of the group identification. The code was maintained at the Data Coordinating Center, University of Texas School of Public Health, Houston. Each vial was thawed immediately at 37 °C for 5 min and the plasma samples were separated by immunoaffinity chromatography followed by ultracentrifugation.

2.3. Immunoaffinity chromatography

We separated plasma into four immunofractions with two-step incubation first with anti-apoE (Perimmune, Inc., Rockville, MD) and second with anti-apoCIII (DMA, Arlington, TX) coupled to Affigel resin. The

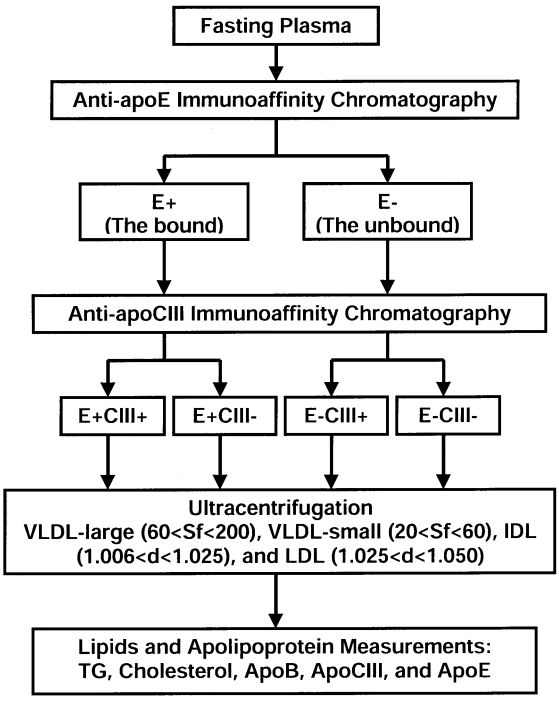

Fig. 1. Summary of laboratory procedures. 1 ml of fasting plasma was incubated with anti-apoE and anti-apoCIII immunoaffinity chromatography in sequence to isolate four types of lipoproteins: E+CIII+, E−CIII+, and E+CIII−, E−CIII−. Each immunofraction was ultracentrifuged to separate large VLDL, small VLDL, IDL, and LDL, then lipids and apolipoproteins were measured.

preparation of resins and validation of the immunoaffinity chromatography procedures were described previously [31]. Fig. 1 shows the flowchart of the immunoaffinity separation. In brief, plasma was incubated at 4 °C overnight with anti-apoE resin, the unbound lipoproteins that were poor in apoE (E−) were collected by gravity flow, and the resin was washed with 5 ml of phosphate buffered-saline (PBS). The bound fractions, lipoproteins that were rich in apoE (E+), were eluted with two separate incubations with 1 ml of 3 M sodium thiocyanate (NaSCN), the first incubation for 3 min and the second incubation for 1.5 min, respectively. NaSCN contained in the E+ fractions was removed immediately by gel filtration (PD-10, Amersham-Pharmacia Biotech, Sweden). The E+ and E− fractions were subsequently incubated with anti-apoCIII immunoaffinity chromatography using the same incubation, elution, and desalting conditions described for anti-apoE immunoaffinity chromatography. As a result, four immunofractions were isolated from the plasma of each patient (E+CIII+, E+CIII−, E−CIII+, and E−CIII−). These plasma fractions were concentrated to 0.6 ml with Ultrafree concentrators (molecular size cutoff, 50 kDa, Millipore, Bedford, MA). For simplicity, we did not report the results for the E+CIII− fraction, which were very low in all subjects as we reported previously for nondiabetic people [31,32]. The recoveries after immunoaffinity chromatography were 87% for cholesterol and 83% for TG.

2.4. Very fast ultracentrifugation

For ultracentrifugation, the following equipment was used: Beckman Optima TLX ultracentrifuge, Beckman TLA 120.2 fixed-angle rotor ($r_{max} = 38.9$ mm, $r_{min} = 24.5$ mm), thick-wall polycarbonate tubes (11×35 mm, No. 343778, without cap, Beckman, Palo Alto, CA). After each centrifugation, the supernatants were collected by aspiration with a glass Pasteur pipette (Fischer, Pittsburgh, PA). Large VLDL ($60 < S_f < 200$), small VLDL ($20 < S_f < 60$), IDL ($1.006 < d < 1.025$ kg/l), and LDL ($1.025 < d < 1.050$) were isolated by stepwise increase of the solute density through the addition of potassium bromide solutions (KBr, SIGMA, St. Louis, MO) with the method by Pietzsch et al. [36] with the following modifications. Densities were adjusted and verified on an optical densitometer (Bausch & Lomb, Rochester, NY). To prepare large VLDL, we transferred 600 μl of the concentrated immunofraction to an ultracentrifugation tube. The sample was overlaid with 400 μl of $d = 1.006$ kg/l and submitted to ultracentrifugation (23 min, 15 °C, $157\,000 \times g$). The large VLDL in the top 400 μl of each tube was carefully harvested. To prepare small VLDL, the remaining solution was carefully mixed by pipetting, overlaid with 400 μl of $d = 1.006$ kg/l, and submitted to ultracentrifugation (1 h 17 min, 15 °C, $537\,000 \times g$). The small VLDL was drawn with the same manner described for the large VLDL. To prepare IDL, we mixed 35.9 μl of 40% saturated KBr solution with 64.1 μl of double distilled water and the 100 μl of mixture was added to the remaining fraction to adjust the density to 1.025 kg/l. The final volume was made up to 1 ml with $d = 1.025$ kg/l. Centrifugation was performed (1 h 51 min, 15 °C, $537\,000 \times g$) and 400 μl of the supernatant, containing IDL, was collected. To prepare LDL, we mixed 52.1 μl of 40% saturated KBr solution with 47.9 μl of double distilled water and added the 100 μl of mixture to the remaining fraction to adjust the density to 1.050 kg/l. After making the final volume up to 1 ml with $d = 1.050$ kg/l, we submitted the sample to ultracentrifugation (2.5 h, 15 °C, $537\,000 \times g$). 400 μl of supernatant containing LDL, was collected. We used $d < 1.025$ for IDL to collect all possible IDL particles and used $d < 1.050$ for LDL to isolate relatively pure LDL, minimizing contamination with lipoprotein (a) and large-size HDL particles. The run time and speeds were standardized by comparison with the classic Lindgren method using a SW41 swinging bucket rotor (Beckman, Palo Alto, CA) so that the lipid measurements of the two ultracentrifuge methods were similar as previously shown [37]. It was shown that this ultracentrifuge method only minimally

affects apolipoproteins of these lipoprotein particles [36].

2.5. Lipid and apolipoprotein measurements

ApoB was measured by sandwich ELISA. The following materials were used: goat polyclonal anti-human apoB (Biogenesis Co., Rockville, MD, coating antibody) and mouse monoclonal anti-human apoB (Chemicon International Inc., Temecula, CA, capture antibody), alkaline phosphatase-linked conjugate (Jackson Immunoresearch Lab Inc., Westgroove, PA) and *p*-nitrophenyl phosphate (PNPP) substrate (Sigma Chemicals), and 3 M NaOH solution for stopping color development. The plate was read at 405 nm. Standards were calibrated to reference plasma provided by the Centers for Disease Control (Atlanta, GA). Cholesterol and TG were measured by enzymatic methods (Cobas MIRA plus, Roche Diagnostic System Inc., Nutley, NJ). ApoCIII and apoE were measured by immunoturbidometry (WACO chemicals USA, Richmond, VA) on the Cobas MIRA plus system. Intra-assay coefficient of variance of cholesterol, TG, apoB, apoCIII, and apoE were 2, 3, 6, 5 and 5%, respectively. Inter-assay coefficient of variance for measurements were 4, 6, 8, 10 and 7%, respectively. Plasma insulin was measured using microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan) at the core laboratory for the CARE trial, Washington University in St. Louis. This method was highly selective for insulin with no cross-reactivity with proinsulin ($< 0.005\%$).

2.6. Statistical analysis

Statistical analyses were performed with SAS software (Version 8.0, SAS Institute, GA). Outlier analysis was performed and no data were excluded. Analysis of variance (ANOVA) and paired group comparisons were performed with the PROC GLM CONTRAST procedure. Since one sample from each of the four groups was included in each run and since the study was completed in 15 runs, the variable 'run' was included to adjust for run-to-run variation. Multiple linear regression analysis was performed to investigate the association of apoCIII containing lipoproteins with plasma TG, HOMA index (homeostasis model assessment = glucose (mmol/l) × insulin (μU/ml)/22.4), and diabetes status. Plasma TG was dichotomized (NTG, if TG < 160 mg/dl, HTG, if TG > 200) for regression analysis. HOMA index was also added as a dichotomized variable based on the cut point for normal and insulin resistant status (0, if HOMA ≤ 2, normal; 1, if HOMA > 2, insulin resistant). BMI, history of hypertension, total cholesterol, exercise, current smoking, and use of oral hypoglycemic medications were covariates. In certain analyses, plasma glucose or insulin concentrations were added together with the other covariates instead of the HOMA index.

3. Results

3.1. Baseline characteristics

Clinical and biochemical characteristics of the patients are shown in Table 1. The TG concentrations in the HTG groups were approximately two times higher than those in the NTG groups but were similar between the two HTG groups or the two NTG groups. The concentrations of LDL cholesterol were similar in the four groups. The concentrations of HDL cholesterol were lower in the HTG groups than in the NTG groups. Groups with diabetes had higher insulin levels than the nondiabetic groups (14.6 vs. 9.1 μU/ml for DM and nonDM group, respectively, $P = 0.03$). The DM-HTG group had the highest insulin level and HOMA index, followed by the DM-NTG group and the nonDM-HTG group. The nonDM-NTG group had the lowest insulin levels and the lowest HOMA index. Age and sex were the matching factors and were similar among the four groups. Characteristics affecting plasma lipid levels such as alcohol consumption, smoking, and use of antihypertensive medications were similar among the four groups. BMI was higher in the DM groups than in the nonDM-NTG groups. The trend for waist circumference was in the same direction as BMI. The NTG groups in both DM and nonDM group exercised more frequently than the HTG groups.

3.2. Lipids and apolipoproteins of TG-rich lipoproteins containing apoCIII

3.2.1. Effects of hypertriglyceridemia

The effects of hypertriglyceridemia were tested by two comparisons: within diabetic groups (DM-NTG vs. DM-HTG) and non-diabetic groups (nonDM-NTG vs. nonDM-HTG). HTG status was associated with higher TG, cholesterol, apoB (Table 2), and apoCIII concentrations (Fig. 2) in large and small VLDL particles that contained apoCIII, in both diabetic and nondiabetic groups. HTG status was also associated with higher apoE concentrations in large VLDL E+CIII+ in patients with and without DM (e.g. 4.91 and 3.94 mg/dl for DM-HTG and DM-NTG, respectively, $P < 0.05$). The HTG groups tended to have higher concentrations of cholesterol and TG in the IDL CIII+ particles (Table 2). There was no difference in LDL concentrations between HTG and NTG groups (data not shown). ApoCIII/B ratio tended to be higher in HTG than NTG groups, although not significantly (Table 3). ApoE/B ratio was similar among the four groups (Table 3).

Table 1
Characteristics of groups with or without diabetes, NTG or HTG ($n = 15$ for each group)

Characteristic	DM-NTG TG < 160	DM-HTG TG ≥ 200	NonDM-NTG TG < 160	NonDM-HTG TG ≥ 200	ANOVA P-value
N	15	15	15	15	
TG (mg/dl)	131 ± 25	232 ± 29	114 ± 27	228 ± 29	< 0.0001
Cholesterol (mg/dl)					
Total	204 ± 14	213 ± 13	199 ± 19	221 ± 12	< 0.001
LDL	140 ± 14	134 ± 14	135 ± 15	142 ± 11	0.36
HDL	38 ± 10	32 ± 5	41 ± 8	34 ± 5	< 0.01
Glucose (mg/dl)	164 ± 49	200 ± 49	89 ± 13	96 ± 7	< 0.0001
Insulin (μU/ml)	13.3 ± 11.9*	15.8 ± 10.8*	7.7 ± 4.6	10.6 ± 8.0	0.07
HOMA index	5.0 ± 4.2	7.7 ± 5.5	1.9 ± 1.3	2.5 ± 1.9	< 0.0001
Age (year)	60 ± 10	60 ± 9	63 ± 7	59 ± 9	0.62
Males	13	14	14	14	0.94
White	12	13	14	13	0.88
Alcohol (drinks/2 weeks)					0.27
None	13	9	8	9	
1–4	2	5	7	6	
5–10	0	1	0	0	
CHF	0	1	1	1	0.90
Hypertension	2	7	7	6	0.24
Diabetes	100	100	0	0	–
Angina	2	3	1	5	0.43
LVEF	52 ± 11	55 ± 9	52 ± 13	58 ± 10	0.52
Exercise (≥ 3 times per week)	10	4	14	8	< 0.01
Current smoker	1	0	3	0	0.12
Blood pressure (mmHg)					
Systolic	127 ± 14	132 ± 14	130 ± 16	137 ± 14	0.27
Diastolic	77 ± 13	77 ± 9	80 ± 10	82 ± 8	0.56
BMI (kg/m^2)	30 ± 6	31 ± 5	26 ± 5	29 ± 3	0.03
Waist circumference (cm)	102 ± 18	99 ± 20	97 ± 15	99 ± 8	0.67
Waist/hip ratio	1.04 ± 0.34	0.90 ± 0.15	0.94 ± 0.07	0.95 ± 0.05	0.20
Antihypertensive drugs[a]	10	11	9	11	0.91
Oral hypoglycemic drugs	11	11	0	0	< 0.0001

Data are the mean ± S.D. or the number of patients, except as indicated. Plasma values (mg/dl or μU/ml), fasting state. CHF, congestive heart failure, LVEF, left ventricular ejection fraction; DM, diabetes mellitus; NTG, normotriglyceridemic; HTG, hypertriglyceridemic; HOMA, homeostasis model assessment = glucose (mmol/l) × insulin (μU/ml)/22.4. Calculated LDL cholesterol.

[a] Patients using beta-blockers or diuretics were excluded.

* $P = 0.03$ for a difference in insulin between the two diabetic and the two nondiabetic groups.

3.2.2. The effects of diabetes status

The effect of diabetes status on TG-rich lipoproteins was compared in normotriglyceridemic groups (DM-NTG vs. nonDM-NTG) and in hypertriglyceridemic groups (DM-HTG vs. nonDM-HTG). Diabetes status was not uniformly associated with the plasma apoB or apoCIII concentrations of apoCIII-containing (apoC-III+) lipoproteins (Table 2, Fig. 2). In no instance was an apoB lipoprotein concentration significantly higher in a DM than a nonDM group. In several instances in the HTG groups, the DM group had even lower concentrations of apoCIII+ lipoproteins than those the nonDM group; cholesterol of E+CIII+ type of small VLDL (1.66 vs. 1.98 mg/dl, $P = 0.02$), apoE of E+ CIII+ type of small VLDL (1.10 vs. 1.32 mg/dl, $P = 0.03$). Both ApoCIII/B and ApoE/B ratios were similar between DM and nonDM groups (Table 3).

3.3. Linear regression analysis

We performed multivariate linear regression analysis to identify the independent variables associated with apoCIII containing particles. The data for all four groups were pooled. The results were similar to those presented for the between-group comparisons in Table 2 and Fig. 2. Plasma total TG concentration was strongly associated with cholesterol and apoB levels in large VLDL E−CIII+ and small VLDL E−CIII+ (e.g. $\beta = 3.00$, $P = 0.01$ for cholesterol of large VLDL E− CIII+). Diabetes status was not associated with higher levels of apoCIII+ particles, but was associated with low levels of cholesterol and apoB in small VLDL E− CIII+ ($\beta = -0.6$, $P = 0.006$). The HOMA index was associated with low levels of cholesterol in small VLDL E−CIII+ ($\beta = -2.5$, $P = 0.053$). Insulin level was not

Table 2
Lipid and apolipoprotein B concentrations

	DM-NTG, TG < 160 $N = 15$	DM-HTG, TG ≥ 200 $N = 15$	NonDM-NTG, TG < 160 $N = 15$	NonDM-HTG, TG ≥ 200 $N = 15$	ANOVA P-value
VLDL-large E+CIII+					
ApoB	2.31±1.08	2.77±1.61**	2.33±1.19	2.50±1.19	0.02
Cholesterol	5.15±2.25	6.38±2.80*	4.89±1.61	7.06±3.38††	0.0005
TG	52.68±24.04	83.23±46.12**	46.41±27.02	85.18±39.61††	<0.0001
VLDL-large E−CIII+					
ApoB	2.87±1.78	2.93±1.97	2.56±1.53	3.52±2.14††	NS
Cholesterol	5.26±2.74	4.65±2.24	3.99±2.79§	6.13±3.72††,¶	0.015
TG	11.25±5.29	14.58±5.40	9.01±4.54	17.23±11.97††	0.0005
VLDL-large E−CIII−					
ApoB	1.80±1.89	1.95±1.42	1.33±1.03	1.73±1.11	NS
Cholesterol	3.09±2.17	3.92±2.69	3.15±2.95	3.55±2.23	NS
TG	7.35±5.11	12.18±7.88**	6.59±4.31	10.56±7.22††	<0.0001
VLDL-small E+CIII+					
ApoB	0.63±0.28	0.76±0.33*	0.64±0.28	0.72±0.33	0.003
Cholesterol	1.38±0.47	1.66±0.53*	1.43±0.46	1.98±0.71††,¶¶	<0.0001
TG	9.27±3.02	11.78±3.54**	9.46±3.19	12.46±4.96††	<0.0001
VLDL-small E−CIII+					
ApoB	0.42±0.33	0.55±0.44	0.40±0.39	0.53±0.33	0.027
Cholesterol	0.77±0.41	0.96±0.97	0.78±0.42	1.13±0.55††	<0.0001
TG	4.63±2.13	6.20±2.13*	4.59±3.42	7.04±3.44††	<0.0001
VLDL-small E−CIII−					
ApoB	1.09±0.69	1.45±0.94	1.43±1.22	1.44±1.03	0.007
Cholesterol	1.10±0.91	1.24±0.97	1.43±1.60	1.67±1.28	0.006
TG	4.57±3.44	5.37±3.84	4.73±3.53	6.17±4.82	0.001
IDL E+CIII+					
ApoB	0.71±0.31	0.71±0.36	0.79±039	0.74±039	NS
Cholesterol	1.48±0.58	1.42±0.68	1.66±1.09	1.67±0.69	NS
Triglyceride	3.15±1.50	4.49±3.85	3.49±1.79	3.84±1.54	NS (0.053)
IDL E−CIII+					
ApoB	1.11±0.81	1.13±0.69	1.11±0.89	1.18±0.64	NS
Cholesterol	1.86±0.81	1.87±0.66	1.96±1.16	2.12±0.61	0.042
Triglyceride	3.47±2.23	3.97±1.72	3.52±2.23	4.17±2.09	0.001
IDL E−CIII−					
ApoB	2.28±1.81	2.93±2.58	2.37±2.17	2.20±2.06	NS
Cholesterol	1.70±1.62	1.85±1.51	2.31±2.29	23.2±2.42	NS
Triglyceride	1.68±1.57	2.63±2.04	2.10±2.20	2.77±2.45	NS

Values are mean±S.D. NS denotes nonsignificance at $P = 0.05$. † $0.05 < P < 0.1$ compared with nonDM-NTG.
** $P < 0.05$ compared with DM-NTG.
* $0.05 < P < 0.1$ compared with DM-NTG.
†† $P < 0.05$ compared with nonDM-NTG
§ $0.05 < P < 0.10$ compared with DM-NTG.
¶ $0.05 < P < 0.10$ compared with DM-HTG.
¶¶ $P < 0.05$ compared with DM-HTG.

significantly associated with any of the apoB lipoprotein types.

4. Discussion

We previously reported that apoCIII concentrations in apoB lipoproteins are considerably stronger risk factors than plasma TG, and, in multivariate analysis, explained the mild risk associated with TG [19]. The specific role of apoCIII in diabetic patients is complicated by a moderate correlation with plasma TG concentration, a relatively weak lipid risk factor with uncertain independence as a risk factor in diabetics [1,2]. The higher TG levels in diabetics could not, per se, account for the higher CHD incidence. For example, the difference in plasma TG between diabetic and nondiabetic people found in the UKPDS [2], corresponds to a 20% increased risk in men and 40% in women, using the univariate relationship between plasma TG and CHD in a meta-analysis [38]. These estimates are halved in multivariate analysis. We aimed to evaluate the specifi-

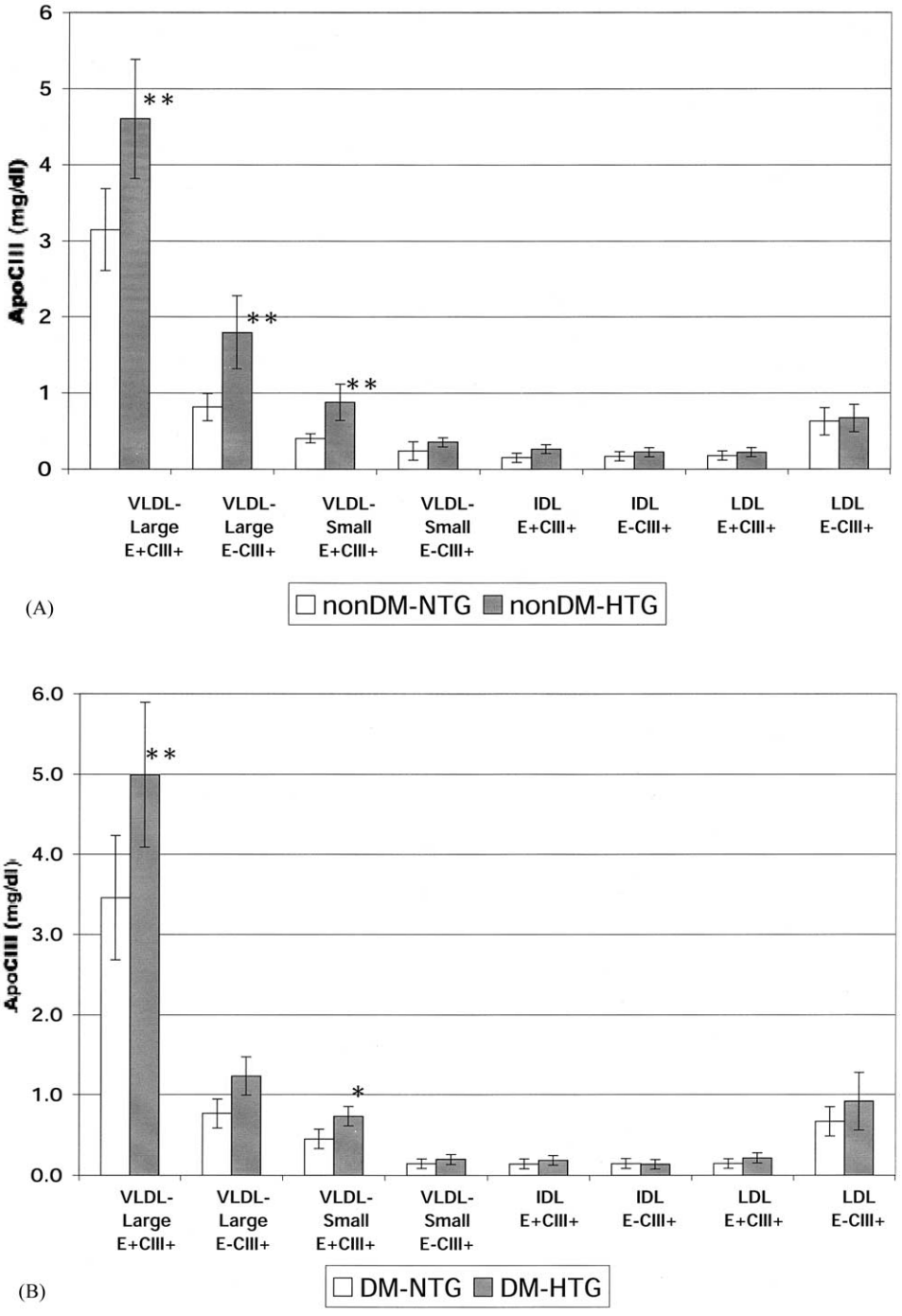

Fig. 2. Apolipoprotein CIII concentrations in hypertriglyceridemia compared with normotriglyceridemia in groups with or without diabetes. Left panel (A) shows nonDM groups. Right panel (B) shows DM groups. **, $P < 0.05$ for NTG vs. HTG. *, $0.05 < P < 0.10$ for NTG vs. HTG. There was no difference between DM and nonDM. Error bars as SDs.

city of involvement of apoCIII in diabetics by matching diabetic and nondiabetic patients for plasma TG concentrations. ApoCIII was associated with plasma TG concentration, as expected. However, contrary to our hypothesis, VLDL, IDL and LDL with apoCIII were similar in diabetic and non-diabetic groups. The HTG groups, diabetic or nondiabetic, had higher concentrations of VLDL with apoCIII than NTG, as previously found in other nondiabetic persons [32].

There is much evidence that apoCIII in apoB lipoproteins is an important component of atherogenic dyslipidemia. Since apoCIII inhibits lipolysis [12–14]

Table 3
Apolipoprotein molar ratios

	DM-NTG	DM-HTG	NonDM-NTG	NonDM-HTG
ApoCIII/ApoB				
VLDL-large E+ CIII+	47±25	56±19	42±25	57±25
VLDL-large E− CIII+	17±8	26±8	20±10	32±7
VLDL-small E+ CIII+	30±16	40±15	26±19	50±16
VLDL-small E− CIII+	21±16	22±9	36±9	42±19
IDL E+CIII+	12±6	16±5	12±11	22±11
IDL E−CIII+	8±5	7±5	9±4	12±5
ApoE/ApoB				
VLDL-large E+ CIII+	28±7	29±10	27±12	31±15
VLDL-small E+ CIII+	22±12	25±9	20±7	33±14
IDL E+CIII+	14±7	17±15	12±4	21±11

Mean±S.D. *P*-values for differences among the groups are all > 0.10.

and apoE-mediated clearance [15–18] of apoB lipoproteins compared with the lipoproteins that do not contain apoCIII, vascular endothelium has a high probability to take up these particles. This mechanism may be similar in patients with and without diabetes. It has been suggested that apoCIII may have a particular role in patients with diabetes, since insulin resistance or insulin deficiency in diabetic patients blunts downregulation of apoCIII expression in the liver [23,24]. Diabetic patients usually have higher levels of apoCIII and plasma TG than controls [39–41]. Dallongeville et al. demonstrated that polymorphism in the insulin response element of the apoCIII gene promoter influences the correlation between insulin and TG or TG-rich lipoproteins in humans [42]. Both VLDL TG and apoCIII production are increased by the liver and by cultured hepatocytes of obese, hyperinsulemic rats [43,44]. ApoCIII production is increased in patients with hypertriglyceridemia [45], although it is unknown whether this is dependent or independent of glycemic or insulinemic status. In the present study, we did not find a relationship between fasting insulin and VLDL apoB or apoCIII concentrations, although the sample size may have limited the ability to detect mild correlations. However, it has not been clearly shown that plasma apoCIII concentrations are higher in patients with diabetes if their TG levels are matched with patients without diabetes. The present study found that the plasma concentration of VLDL particles that contain apoCIII and the apoCIII content of these particle types are strongly associated with fasting TG levels, not with diabetes status per se. This agrees with the report by Ishibashi et al. [46] that the apoCIII content of VLDL was similar in diabetics and nondiabetics. This suggests that mechanisms that establish hypertriglyceridemia and atherogenic dyslipidemia may be similar in patients with and without diabetes.

In conclusion, our findings suggest that fasting TG level is a key determinant for apoCIII concentrations in TG rich lipoproteins, regardless of diabetes status. Although it is likely that these TG-rich lipoproteins contribute to the high incidence of CHD in patients with diabetes, diabetes status per se does not appear to be associated with high concentrations of apoCIII-containing TG-rich lipoprotein particles, if their plasma TG levels are similar.

Acknowledgements

The authors are thankful to Dr Eric Rimm for his contributions to the development of this research and his continued support as a doctoral thesis committee member for Dr Lee. This work was supported by an investigator-initiated grant from Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ.

References

[1] Fontbonne A, Eschwege E, Cambien F, Richard JL, Ducimetiere P, Thibult N, Warnet JM, Claude JR, Rosselin GE. Hypertriglyceridaemia as a risk factor of coronary heart disease mortality in subjects with impaired glucose tolerance or diabetes. Results from the 11-year follow-up of the Paris Prospective Study. Diabetologia 1989;32:300–4.
[2] Turner RC, Millns H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR. Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). Br Med J 1998;316:823–8.
[3] Syvanne M, Taskinen MR. Lipids and lipoproteins as coronary risk factors in non-insulin-dependent diabetes mellitus. Lancet 1997;350(Suppl. 1):SI20–3.
[4] Taskinen MR, Packard CJ, Shepherd J. Effect of insulin therapy on metabolic fate of apolipoprotein B-containing lipoproteins in NIDDM. Diabetes 1990;39:1017–27.
[5] Steiner G, Lewis GF. Hyperinsulinemia and triglyceride-rich lipoproteins. Diabetes 1996;45(Suppl. 3):S24–6.
[6] Malmstrom R, Packard CJ, Caslake M, Bedford D, Stewart P, Yki-Jarvinen H, Shepherd J, Taskinen MR. Defective regulation of triglyceride metabolism by insulin in the liver in NIDDM. Diabetologia 1997;40:454–62.
[7] Verges BL. Dyslipidaemia in diabetes mellitus. Review of the main lipoprotein abnormalities and their consequences on the development of atherogenesis. Diabetes Metab 1999;25(Suppl. 3):32–40.
[8] Howard BV, Reitman JS, Vasquez B, Zech L. Very-low-density lipoprotein triglyceride metabolism in non-insulin-dependent diabetes mellitus. Relationship to plasma insulin and free fatty acids. Diabetes 1983;32:271–6.
[9] Venkatesan S, Imrie H, Read S, Halliday D. Apo C subclasses from non-insulin-dependent diabetic patients—a quantitative comparison with control subjects. Biochem Soc Trans 1995;23:278S.

[10] Bren ND, Rastogi A, Kottke BA. Quantification of human plasma apolipoproteins C-I, C-II, and C-III by radioimmunoassays. Mayo Clin Proc 1993;68:657–64.
[11] Briones ER, Mao SJ, Palumbo PJ, O'Fallon WM, Chenoweth W, Kottke BA. Analysis of plasma lipids and apolipoproteins in insulin-dependent and noninsulin dependent diabeties. Metabolism 1984;33:42–9.
[12] McConathy WJ, Gesquiere JC, Bass H, Tartar A, Fruchart JC, Wang CS. Inhibition of lipoprotein lipase activity by synthetic peptides of apolipoprotein C-III. J Lipid Res 1992;33:995–1003.
[13] Ginsberg HN, Le NA, Goldberg IJ, Gibson JC, Rubinstein A, Wang-Iverson P, Norum R, Brown WV. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. J Clin Invest 1986;78:1287–95.
[14] Wang CS, McConathy WJ, Kloer HU, Alaupovic P. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. J Clin Invest 1985;75:384–90.
[15] Aalto-Setala K, Weinstock PH, Bisgaier CL, Wu L, Smith JD, Breslow JL. Further characterization of the metabolic properties of triglyceride-rich lipoproteins from human and mouse apoC-III transgenic mice. J Lipid Res 1996;37:1802–11.
[16] Agnani G, Bard JM, Candelier L, Delattre S, Fruchart JC, Clavey V. Interaction of LpB, LpB:E, LpB:C-III, and LpB:C-III:E lipoproteins with the low density lipoprotein receptor of HeLa cells. Arterioscler Thromb 1991;11:1021–9.
[17] Ebara T, Ramakrishnan R, Steiner G, Shachter NS. Chylomicronemia due to apolipoprotein CIII overexpression in apolipoprotein E-null mice. Apolipoprotein CIII-induced hypertriglyceridemia is not mediated by effects on apolipoprotein E. J Clin Invest 1997;99:2672–81.
[18] Sehayek E, Eisenberg S. Mechanisms of inhibition by apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride-rich lipoproteins through the low density lipoprotein receptor pathway. J Biol Chem 1991;266:18259–67.
[19] Sacks FM, Alaupovic P, Moye LA, Cole TG, Sussex B, Stampfer MJ, Pleffer MA, Braunwald E. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. Circulation 2000;102:1886–92.
[20] Luc G, Fievet C, Arveiler D, Evans AE, Bard JM, Cambien F, Fruchart JC, Ducimetiere P. Apolipoproteins C-III and E in apoB- and non-apoB-containing lipoproteins in two populations at contrasting risk for myocardial infarction: the ECTIM study. Etude Cas Temoins sur 'Infarctus du Myocarde. J Lipid Res 1996;37:508–17.
[21] Hodis HN, Mack WJ, Azen SP, Alaupovic P, Pogoda JM, LaBree L, Hemphill LC, Kramsch DM, Blankenhorn DH. Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovasiatin. Circulation 1994;90:42–9.
[22] Gervaise N, Garrigue MA, Lasfargues G, Lecomte P. Triglycerides, apo C3 and LpB:C3 and cardiovascular risk in type II diabetes. Diabetologia 2000;43:703–8.
[23] Chen M, Breslow JL, Li W, Leff T. Transcriptional regulation of the apoC-III gene by insulin in diabetic mice: correlation with changes in plasma triglyceride levels. J Lipid Res 1994;35:1918–24.
[24] Reddy S, Yang W, Taylor DC, Shen X, Oxender D, Kust G, Leff T. Mitogen-activated protein kinase regulates transcription of the ApoCIII gene. Involvement of the orphan nuclear receptor HNF4. J Biol Chem 1999;274:33050–6.
[25] Pan XR, Cheung MC, Walden CE, Hu SX, Bierman EL, Albers JJ. Abnormal composition of apoproteins C-I, C-II, and C-III in plasma and very-low-density lipoproteins of non-insulin-dependent diabetic Chinese. Clin Chem 1986;32:1914–20.
[26] Weisgraber KH, Innerarity TL, Harder KJ, Mahley RW, Milne RW, Marcel YL, Sparrow JT. The receptor binding domain of human apolipoprotein E. Monoclonal inhibition of binding. J Biol Chem 1983;258:12348–54.
[27] Beisiegel U, Weber W, Ihrke G, Herz J, Stanley KK. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. Nature 1989;341:162–4.
[28] Syvanne M, Rosseneu M, Labeur C, Hilden H, Taskinen MR. Enrichment with apolipoprotein E characterizes postprandial TG-rich lipoproteins in patients with non-insulin-dependent diabetes mellitus and coronary artery disease; a preliminary report. Atherosclerosis 1994;105:25–34.
[29] Chivot L, Mainard F, Bigot E, Bard JM, Auget JL, Madec Y, Fruchart JC. Logistic discriminant analysis of lipids and apolipoproteins in a population of coronary bypass patients and the significance of apolipoproteins C-III and E. Atherosclerosis 1990;82:205–11.
[30] Genest JJ, Jr, Bard JM, Fruchart JC, Ordovas JM, Wilson PF, Schaefer EJ. Plasma apolipoprotein A-I, A-II, B, E and C-III containing particles in men with premature coronary artery disease. Atherosclerosis 1991;90:149–57.
[31] Khoo C, Campos H, Judge H, Sacks FM. Effects of estrogenic oral contraceptives on the lipoprotein B particle system defined by apolipoproteins E and C-III content. J Lipid Res 1999;40:202–12.
[32] Campos H, Perlov D, Khoo C, Sacks FM. Distinct patterns of lipoproteins with apoB defined by presence of apoE or apoC-III in hypercholesterolemia and hypertriglyceridemia. J Lipid Res 2001;42:1239–49.
[33] Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR, Braunwald E. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. New Engl J Med 1996;335:1001–9.
[34] Sacks FM, Rouleau JL, Moye LA, Pfeffer MA, Warnica JW, Arnold JM, Nash DT, Brown LE, Sestier F, Rutherford J, et al. Baseline characteristics in the Cholesterol and Recurrent Events (CARE) trial of secondary prevention in patients with average serum cholesterol levels. Am J Cardiol 1995;75:621–3.
[35] Goldberg RB, Mellies MJ, Sacks FM, Moye LA, Howard BV, Howard WJ, Davis BR, Cole TG, Pfeffer MA, Braunwald E. Cardiovascular events and their reduction with pravastatin in diabetic and glucose-intolerant myocardial infarction survivors with average cholesterol levels: subgroup analyses in the cholesterol and recurrent events (CARE) trial. Circulation 1998;98:2513–9.
[36] Pietzsch J, Subat S, Nitzsche S, Leonhardt W, Schentke KU, Hanefeld M. Very fast ultracentrifugation of serum lipoproteins: influence on lipoprotein separation and composition. Biochim Biophys Acta 1995;1254:77–88.
[37] Brousseau T, Clavey V, Bard JM, Fruchart JC. Sequential ultracentrifugation micromethod for separation of serum lipoproteins and assays of lipids, apolipoproteins, and lipoprotein particles. Clin Chem 1993;39:960–4.
[38] Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996;3:213–9.
[39] Al-Muhtaseb N, Al-Yusef AR, Bajaj JS. Lipoprotein lipids and apolipoproteins (AI, AII, B, CII, CIII) in type 1 and type 2 diabetes mellitus in young Kuwaiti women. Diabet Med 1991;8:732–7.
[40] Castro Cabezas M, de Bruin TW, de Valk HW, Shoulders CC, Jansen H, Willem Erkelens D. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating

hepatic apolipoprotein B overproduction and insulin resistance. J Clin Invest 1989;92:160–8.
[41] Ikeda T, Ohtani I, Fujiyama K, Hoshino T, Tanaka Y, Tekeuchi T, Mashiba H. Apolipoprotein levels in non-insulin-dependent diabetes mellitus with clinical macroangiapathy. Diabete Metab 1991;17:373–8.
[42] Dallongeville JMA, Cottel D, Fruchart JC, Amouyel P, Helbecque N. Polymorphisms in the insulin response element of APOC-III gene promoter influence the correlation between insulin and triglycerides or triglyceride-rich lipoproteins in humans. Int J Obes Relat Metab Disord 2001;25:1012–7.
[43] Elam MB, Wilcox HG, Cagen LM, Deng X, Raghow R, Kumar P, Heimberg M, Russell JC. Increased hepatic VLDL secretion, lipogenesis, and SREBP-1 expression in the corpulent JCR:LA-cp rat. J Lipid Res 2001;42:2039–48.
[44] Vance JE, Russell JC. Hypersecretion of VLDL, but not HDL, by hepatocytes from the JCR:LA-corpulent rat. J Lipid Res 1990;31:1491–501.
[45] Batal R, Tremblay M, Barrett PH, Jacques H, Fredenrich A, Mamer O, Davignon J, Cohn JS. Plasma kinetics of apoC-III and apoE in normolipidemic and hypertriglyceridemic subjects. J Lipid Res 2000;41:706–18.
[46] Ishibashi S, Yamada N, Shimano H, Takaku F, Akanuma Y, Murase T. Composition of very-low-density lipoproteins in non-insulin-dependent diabetes mellitus. Clin Chem 1989;35:808–12.