Lipoprotein Distribution of Apolipoprotein C-III and Its Relationship to the Presence in Plasma of Triglyceride-Rich Remnant Lipoproteins

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The distribution of apolipoprotein C-III (apoC-III) between high-density lipoprotein (HDL) and apoB-containing lipoproteins has been used in lipid-lowering angiographic trials to establish a link between impaired triglyceride (TG)-rich lipoprotein (TRL) metabolism and the progression of coronary artery disease. To investigate the extent to which plasma lipoprotein apoC-III levels reflect the presence in plasma of potentially atherogenic remnant lipoproteins, we studied 4 groups of subjects: (1) normolipidemic (NL, n = 10), (2) hypercholesterolemic (HC, type IIa, low-density lipoprotein cholesterol [LDL-C] > 3.4 mmol/L, n = 10), (3) hypertriglyceridemic (HTG, type IV, TG > 2.3 mmol/L, n = 10), and (4) combined hyperlipidemic (CHL, type IIb, TG > 2.3 mmol/L, LDL-C > 3.4 mmol/L, n = 10). The apoC-III level was measured in plasma lipoproteins separated either by density (ultracentrifugation) or by size (fast protein liquid chromatography [FPLC]), and was compared with 4 parameters reflecting remnant lipoprotein levels (ie, very–low-density lipoprotein cholesterol [VLDL-C], intermediate-density lipoprotein cholesterol [IDL-C], remnant-like particle cholesterol [RLP-C], and intermediate-sized lipoprotein [ISL] apoE). Our results demonstrate that (1) increased amounts of apoC-III associated with plasma VLDL, TRL, or apoB-containing lipoproteins (LpB), as well as increased levels of TRL remnant lipoproteins, are a characteristic of HTG patients rather than patients with increased LDL, and (2) plasma levels of apoC-III in VLDL, TRL, or LpB, as well as the HDL apoC-III to LpB apoC-III ratios, are strongly correlated with circulating levels of TRL, although these apoC-III parameters more closely reflect the balance between TRL TG production and lipolysis than the extent of plasma TRL remnant accumulation.

APOLIPOPROTEIN C-III (apoC-III) plays a central role in regulating the plasma metabolism of triglyceride (TG)-rich lipoproteins (TRL) by acting as an inhibitor of lipoprotein lipase[1-3] and of TRL remnant uptake by hepatic lipoprotein receptors.[4-6] ApoC-III thus has the potential to decrease TRL hydrolysis, delay the clearance of TRL remnants, and cause an accumulation in plasma of large TRL and/or smaller more atherogenic TRL remnants. Patients with hypertriglyceridemia (HTG) have increased plasma levels of total apoC-III and an increased proportion of plasma apoC-III associated with TRL.[7-9] Based on the concept that apoC-III is transferred from TRL to high-density lipoproteins (HDL) during TRL lipolysis[10] and that increased levels of TRL favor the exchange of apoC-III from HDL to TRL,[11] it has been proposed that the efficiency of TRL metabolism can be assessed by measuring the relative distribution of apoC-III between HDL and apoB-containing lipoproteins.[12,13] ApoC-III has therefore been quantified in plasma samples subjected to heparin precipitation, with apoC-III in the heparin precipitate being considered to be that of apoB-containing lipoproteins, while that in the heparin supernate is taken to represent HDL apoC-III. The percentage of plasma apoC-III in HDL is inversely correlated with the total plasma or serum TG concentration, and the ratio of heparin supernate apoC-III to heparin precipitate apoC-III is as high as 4.5 in normolipidemic (NL) women (TG ~ 100 mg/dL) and as low as 0.08 in patients with type V hyperlipoproteinemia ([HLP] TG ~ 2,000 mg/dL).[12]

The distribution of apoC-III between HDL and apoB-containing lipoproteins has been used in two lipid-lowering angiographic trials, the Cholesterol Lowering Atherosclerosis Study (CLAS) and the Monitored Atherosclerosis Regression Study (MARS), to establish a link between impaired TRL metabolism and the progression of coronary artery atherosclerosis.[14,15] In the CLAS, the on-trial concentration of non-HDL cholesterol (non-HDL-C) was the only independent predictor of coronary artery disease progression in the placebo group, while in the treated (colestipol plus niacin) group, the only independent predictor of progression was heparin supernate (HDL) apoC-III.[16] In MARS, multivariate analysis showed that apoC-III in the heparin precipitate (very–low-density lipoproteins [VLDL] plus low-density lipoproteins [LDL]) in lovastatin-treated patients was the only factor independently associated with the progression of mild to moderate lesions (<50% stenosis).[17] CLAS and MARS have also provided evidence that apoC-III parameters are linked to an increase in carotid artery intima-media thickness.[18,19]

One explanation as to why lipoprotein apoC-III parameters are related to the development of atherosclerosis is that decreased levels of HDL apoC-III, and increased levels of apoC-III in apoB-containing lipoproteins, reflect the presence of TRL remnants in plasma. Both clinical and experimental evidence supports the concept that remnant lipoproteins are potentially atherogenic.[20] However, the relationship between plasma lipoprotein apoC-III distribution and the accumulation in plasma of remnant lipoproteins has not been previously investigated. The aim of the present study was therefore to determine (1) to what extent HTG and/or hypercholesterolemia (HC) affect the plasma lipoprotein concentration and distribution of apoC-III, (2) to what extent HTG and/or HC affect the plasma concentration of TRL remnants, and (3) which apoC-III parameters are most strongly related to remnant lipoprotein accumulation. To

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answer these questions, we obtained plasma samples from 4 groups of individuals, (1) NL subjects (n = 10), (2) HC patients (type IIa HLP, n = 10), (3) HTG patients (type IV HLP, n = 10), and (4) patients with combined hyperlipidemia ([CHL] type IIb HLP, n = 10), who were selected such that the HC and CHL groups had similar mean LDL-C concentrations and the HTG and CHL groups had similar mean total plasma TG concentrations. We measured apoC-III in plasma lipoproteins separated either by density (ultracentrifugation) or by size (automated gel filtration chromatography [FPLC]) and we compared these measures with 4 parameters reflecting the plasma concentration of potentially atherogenic remnant lipoproteins.

SUBJECTS AND METHODS

Subjects

Healthy male NL subjects (n = 10) were recruited from the staff of our research laboratory, having a plasma TG concentration less than 2.3 mmol/L and LDL-C less than 3.4 mmol/L. Hyperlipidemic patients were selected from those attending our Lipid Clinic at the Clinical Research Institute of Montreal. Patients were selected for Fredrickson[21] type IIa HLP (plasma TG < 2.3 mmol/L, LDL-C > 3.4 mmol/L, n = 10), type IIb HLP (plasma TG > 2.3 mmol/L, LDL-C > 3.4 mmol/L, n = 10), or type IV HLP (plasma TG > 2.3 mmol/L, LDL-C < 3.4 mmol/L, n = 10). Six type IIa patients (statin therapy), 2 type IIb patients (statin or fibrate therapy), and 2 type IV patients (fibrate therapy) were on treatment with lipid-lowering medications (results were not different for these patients versus other patients in the same group). The mean age for the patient groups was not significantly different, and the body mass index was lower in NL compared with hyperlipidemic patients (Table 1). All patients had an apoE 3/3 phenotype, except for 2 NL patients with an apoE 3/2 phenotype, 1 type IIb patient with an apoE 3/2 phenotype, and 5 type IV patients with an apoE 3/2 phenotype. Patients were selected so that the 2 normotriglyceridemic groups had similar mean levels of plasma TG. In the same way, the 2 HTG groups had similarly elevated mean levels of plasma TG. Mean LDL-C concentrations for the NL and type IV groups and the IIa and IIb groups were also similar by selection. Mean plasma lipid and apolipoprotein concentrations for the study groups are shown in Table 1.

Separation of Plasma Lipoproteins

Blood samples were obtained from subjects who had fasted for 12 hours overnight. The blood was drawn under vacuum from an arm vein into tubes containing EDTA (final concentration, 1.5 mg/mL). Plasma was obtained by centrifugation (15 minutes at 3,000 rpm at 4°C) and stored at 4°C until lipids and lipoproteins were analyzed. Remnant-like particle (RLP) and ultracentrifugal fractions were separated within 2 days of plasma isolation. VLDL were separated by ultracentrifugation (UTC) from 2.5 mL plasma, which was centrifuged overnight at density (d) = 1.006 g/mL (50,000 rpm for 10 hours at 4°C). A separate 2.5-mL plasma aliquot was adjusted to d = 1.019, and VLDL plus intermediate-density lipoproteins (IDL) were separated at d = 1.019 g/mL under the same conditions (50,000 rpm for 10 hours at 4°C) in a 50.4 Ti rotor (Beckman Instruments, Palo Alto, CA).

Plasma TRL, intermediate-sized lipoprotein (ISL), and HDL were separated by automated gel filtration chromatography on a fast protein liquid chromatography (FPLC) system as previously described.[22] Briefly, 1-mL plasma samples were manually transferred to a 2-mL sample loop with 2 (0.25 mL) washes with 0.15 mol/L NaCl buffer (0.01% EDTA and 0.02% sodium azide, pH 7.4). The system was programed (Liquid Chromatography Controller LCC-500 Plus, Pharmacia LKB Biotechnology, Uppsala, Sweden) to load and separate samples on a 50-cm column (16 mm ID) packed with cross-linked agarose gel (Superose 6 prep grade; Pharmacia, Uppsala, Sweden). The column was eluted with saline solution at a flow rate of 1.0 mL/min, and 25 minutes after addition of the sample, 90-mL fractions were collected sequentially. Sample elution was monitored spectrophotometrically at optical density 280 nm. TRL-, ISL-, and HDL-containing fractions were pooled according to the optical density elution profile. We have previously shown that apoE, which elutes as a distinct fraction intermediate in size between FPLC-isolated TRL and HDL (ie, ISL), represents apoE associated with remnant lipoproteins.[22] These lipoproteins coelute with LDL-sized lipoproteins. ApoC-III concentrations were determined in TRL, ISL, and HDL pooled fractions. ApoC-III in FPLC-isolated HDL was labeled as HDL_{fplc} apoC-III.

Remnant-like particles were isolated by immunoaffinity chromatography from fresh plasma using RLP cholesterol assay kits (Jimro-II; Japan Immunoresearch Laboratories, Japan). According to the manufacturer's instructions, 5 μL plasma was added to 300 μL gel suspension consisting of anti–human apoA-I and apoB-100 mouse monoclonal antibodies bound to Sepharose. All plasma lipoproteins except those resembling remnants bound to the gel, allowing cholesterol and TG to be determined in the unbound RLP fraction. This fraction contained an apoE-rich population of remnant-like VLDL containing apoB-100, as well as TRL containing apoB-48.[23,24] The suspension was gently mixed for 2 hours at room temperature with a vertical magnetic-bead oscillator (RLP Mixer J-100A, Photal; Otsuka Electronics, Osaka, Japan). The mixture was allowed to settle for 15 minutes, and 230 μL of the supernatant, containing unbound RLP, was transferred for storage at 4°C before determination of RLP lipids.

Table 1. Characteristics of NL and HLP Subjects

Characteristic	NL (n = 10)	HLP		
		Type IIa (n = 10)	Type IIb (n = 10)	Type IV (n = 10)
Age (yr)	42.7 ± 5.1	44.6 ± 5.9	46.4 ± 4.2	52.9 ± 4.1
Body mass index (kg/m^2)	24.5 ± 0.8	28.2 ± 1.5	28.8 ± 1.7	28.9 ± 1.1§
Plasma TG	1.15 ± 0.15	1.17 ± 0.10	3.86 ± 0.36†	3.71 ± 0.39‖
Plasma cholesterol	4.70 ± 0.25	6.32 ± 0.11	7.41 ± 0.15†	5.95 ± 0.45‡
LDL-C	2.98 ± 0.24	4.65 ± 0.09	4.67 ± 0.21	2.99 ± 0.20
HDL-C	1.36 ± 0.10	1.31 ± 0.08	1.01 ± 0.08*	0.97 ± 0.10‡
ApoB	104.2 ± 9.4	149.9 ± 7.2	174.4 ± 7.6*	137.9 ± 6.2§
ApoA-I	136.1 ± 4.5	127.0 ± 6.9	135.9 ± 6.6	125.1 ± 9.1

NOTE. Values represent the mean ± SE. Plasma lipids are expressed in mmol/L; apolipoprotein concentrations are in mg/dL.

* $P < .05$, † $P < .001$ *v* type IIa by unpaired *t* test.

‡ $P < .05$, § $P < .01$, ‖ $P < .001$ *v* NL by unpaired *t* test.

Quantitation of Lipids and Apolipoproteins

Plasma and lipoprotein cholesterol and TG were determined enzymatically on an autoanalyzer (Cobas Mira; Roche, Basel, Switzerland). ApoC-III in total plasma and lipoprotein fractions was determined by in-house enzyme-linked immunosorbent assays.[9] HDL-C and apoC-III were determined by measuring cholesterol and apoC-III levels in the supernatant of 1 mL plasma after precipitation of apoB-containing lipoproteins with heparin-manganese.[25] This HDL apoC-III measurement was labeled as HDL_{prec} apoC-III. VLDL lipid and apoC-III were determined by measuring lipid and apolipoprotein concentrations in the $d < 1.006$ g/mL fraction and were corrected to produce 100% UTC recovery (eg, corrected VLDL apoC-III = measured VLDL apoC-III × [total plasma apoC-III] ÷ [$d < 1.006$ apoC-III + $d > 1.006$ apoC-III]). Enrichment of VLDL with cholesterol was determined by dividing the concentration of VLDL-C by that of VLDL-TG (VLDL-C/VLDL-TG) or dividing the concentration of VLDL-C by that of plasma TG (VLDL-C/plasma TG). IDL lipid and apoC-III concentrations were determined by subtracting lipid and apoC-III measurements for the $d > 1.019$ g/mL fraction from those for the $d > 1.006$ g/mL fraction (both corrected for recovery). LDL-C and LDL apoC-III were determined by subtracting HDL-C and HDL apoC-III from $d > 1.019$ g/mL cholesterol and apoC-III (corrected for recovery). Since the amount of apoC-III in IDL and LDL was small, they were added and expressed as apoC-III in IDL/LDL ($1.006 < d < 1.063$ g/mL). The amount of apoC-III associated with apoB-containing lipoproteins (LpB_{prec} or LpB_{fplc} apoC-III) was calculated by subtracting the HDL apoC-III measured in the supernatant after precipitation (HDL_{prec} apoC-III) or after FPLC separation (HDL_{fplc} apoC-III) from the total plasma apoC-III. The ratio of HDL apoC-III to LpB apoC-III (ratio apoC-III_{prec} or ratio apoC-III_{fplc}) was calculated with lipoprotein isolated by precipitation and by FPLC, respectively. Plasma apoA-I and apoB concentrations were determined by nephelometry (Behring Nephelometer 100 Analyser; Behring, Marburg, Germany). ApoE phenotypes were determined by immunoblotting of plasma separated by isoelectric focusing minigel electrophoresis.[26]

Statistical Analysis

Statistical analyses were performed with SigmaStat statistical software (Jandel, San Rafael, CA). The data are expressed as the mean ± SE. Student's unpaired and paired *t* tests were used for comparisons between groups or within the same group. Pearson correlation coefficients (*r*) were determined to assess relationships between apoC-III and plasma remnant lipoprotein parameters. Differences with a *P* value less than .01 were considered statistically significant.

RESULTS

Plasma lipid and apolipoprotein concentrations for NL and HLP subjects are shown in Table 1. Plasma TG concentrations of type IIb and type IV HLP subjects, according to patient selection, were not significantly different ($P = .94$) and were approximately 3-fold higher than those of NL and type IIa subjects. As expected, HTG subjects had significantly lower HDL-C (though not apoA-I) levels. HC subjects (type IIa and type IIb), also by selection, had similar mean levels of LDL-C, as did type IV and NL subjects. Higher LDL-C levels in HC subjects were associated with higher levels of both total cholesterol and apoB compared with normal and type IV subjects.

Total plasma and lipoprotein apoC-III concentrations of NL and HLP subjects are shown in Table 2. ApoC-III levels are shown for lipoproteins (VLDL, IDL/LDL, and HDL) separated by density (ie, UTC and precipitation) or by size (ie, FPLC). HTG subjects (type IIb and type IV) had significantly higher levels (2-fold) of total apoC-III, VLDL apoC-III (4-fold), and TRL (6-fold) compared with NL and type IIa subjects. They also had higher levels of IDL/LDL, ISL, LpB_{prec}, and LpB_{fplc} apoC-III. Increased levels of LDL-C in type IIa versus NL subjects were associated with significantly higher levels of IDL/LDL apoC-III ($P < .05$) and ISL apoC-III ($P < .05$), but increased levels of LDL-C in type IIb versus type IV were not. HDL apoC-III to LpB apoC-III ratios (prec and/or fplc) were

Table 2. Total Plasma and Lipoprotein ApoC-III Concentrations in NL and HLP Subjects

Parameter	NL (n = 10)	HLP		
		Type IIa (n = 10)	Type IIb (n = 10)	Type IV (n = 10)
Total apoC-III	12.21 ± 1.29	14.79 ± 0.85	29.15 ± 4.13†	30.19 ± 3.66¶
VLDL apoC-III	4.88 ± 0.87	4.92 ± 0.45	19.11 ± 2.77‡	21.58 ± 3.62¶
IDL/LDL apoC-III	1.12 ± 0.58	2.78 ± 0.53	3.63 ± 1.31	3.34 ± 0.58§
HDL_{prec} apoC-III	6.22 ± 0.52	7.09 ± 0.85	6.41 ± 0.87	5.27 ± 0.68
LpB_{prec} apoC-III	5.99 ± 1.29	7.70 ± 0.65	22.74 ± 3.48‡	24.92 ± 3.74¶
Ratio apoC-III_{prec}	1.32 ± 0.30	1.05 ± 0.21	0.32 ± 0.06†	0.26 ± 0.05‖
TRL apoC-III	1.93 ± 0.41	2.01 ± 0.33	12.77 ± 2.54‡	13.46 ± 3.04‖
ISL apoC-III	3.37 ± 0.58	4.67 ± 0.36	9.01 ± 1.21†	9.31 ± 1.08¶
HDL_{fplc} apoC-III	6.85 ± 0.63	7.60 ± 0.67	7.36 ± 0.95	7.17 ± 0.95
LpB_{fplc} apoC-III	5.30 ± 0.90	6.68 ± 0.54	21.79 ± 3.56‡	22.76 ± 3.49¶
Ratio apoC-III_{fplc}	1.56 ± 0.23	1.19 ± 0.13	0.38 ± 0.05‡	0.36 ± 0.06¶
ApoC-III significant difference#				
VLDL *v* TRL	$P < .001$	$P < .001$	$P < .001$	$P < .001$
IDL/LDL *v* ISL	$P < .01$	$P < .001$	$P < .01$	$P < .001$
HDL_{prec} *v* HDL_{fplc}	—	—	—	$P < .01$
LpB_{prec} *v* LpB_{fplc}	—	$P < .01$	—	$P < .01$

NOTE. Values represent the mean ± SE and are expressed in mg/dL.

*$P < .05$, †$P < .01$, ‡$P < .001$ *v* type IIa by unpaired *t* test.

§ $P < .05$, ‖$P < .01$, ¶$P < .001$ *v* NL by unpaired *t* test.

Statistical comparison (by paired *t* test) between apoC-III levels in lipoproteins isolated either by ultracentrifugation/precipitation or by FPLC.

Table 3. Correlation Between Plasma ApoC-III Parameters and Plasma Lipid, Lipoprotein, and Apolipoprotein Levels

Parameter	Cholesterol	TG	VLDL-TG	LDL-C	HDL-C	ApoA-I	ApoB
Total apoC-III	.568†	.867†	.872†	—	−.458*	—	—
HDL_{prec} apoC-III	—	—	—	—	.457*	.695†	—
LpB_{prec} apoC-III	.542†	.908†	.919†	—	−.527†	—	—
Ratio apoC-III_{prec}	−.384	−.605†	−.552†	—	.525†	—	−.421*
HDL_{fplc} apoC-III	—	—	—	—	—	.493†	—
LpB_{fplc} apoC-III	.545†	.923†	.931†	—	−.554†	—	—
Ratio apoC-III_{fplc}	−.484*	−.704†	−.714†	—	.601†	—	−.489*

NOTE. Values represent statistically significant correlations(r) for all subjects combined (N = 40): *$P < .01$, †$P < .001$. Correlation coefficients that are not significant are not shown.

significantly (3-fold, $P < .01$) lower in HTG versus NL subjects. No significant differences were observed in HDL apoC-III levels between groups whether HDL was isolated by UTC/precipitation or by gel filtration. In all groups, VLDL apoC-III levels were significantly higher than TRL apoC-III ($P < .001$), and conversely, IDL/LDL apoC-III levels were significantly lower than ISL apoC-III ($P < .01$). Mean HDL_{prec} apoC-III levels tended to be lower than HDL_{fplc} apoC-III (~10% in NL and type IIa and ~25% in type IIb and type IV patients), and subsequently, LpB_{prec} apoC-III levels tended to be higher than LpB_{fplc} apoC-III (reaching statistical significance, $P < .01$, in type IIa and type IV groups).

To determine the relationship between HTG, HC, and apoC-III parameters, linear regression analysis was performed and correlation coefficients were determined (Table 3). The most striking feature of this analysis is the lack of any statistically significant correlation between LDL-C levels and apoC-III parameters. In contrast, total TG and VLDL-TG levels were significantly correlated with total apoC-III, LpB_{prec} apoC-III, and LpB_{fplc} apoC-III and inversely correlated ($P < .001$) with apoC-III ratio measurements. The correlation coefficient (r) between VLDL apoC-III and VLDL-TG was .951 ($P < .001$). Weaker but statistically significant correlations were observed between total cholesterol and apoC-III parameters and between HDL-C and apoC-III parameters (Table 3). Significant correlations were found between HDL apoC-III levels (prec and fplc) and other HDL parameters (HDL-C and apoA-I), but interestingly, HDL apoC-III was not significantly correlated with total cholesterol, total TG, VLDL-TG, or LDL-C.

Parameters reflecting the presence in plasma of TRL remnant lipoproteins are shown for 4 groups of subjects in Table 4. Plasma TRL remnant lipoprotein parameters (except IDL-TG) were significantly higher (2 to 5 times) in HTG compared with normotriglyceridemic subjects. No differences were observed between type IIb and type IV subjects, nor between NL and type IIa patients. VLDL fractions of type IIb and type IV were enriched in cholesterol (compared with NL and type IIa, respectively), as reflected by significantly higher VLDL-C/VLDL-TG and VLDL-C/plasma TG ratios. The same was true for IDL in the 2 HTG groups. In type IIb and type IV patients, IDL fractions contained approximately 2 times more cholesterol than TG, whereas RLP fractions contained 2 times more TG than cholesterol. In all groups, IDL and RLP fractions were enriched in cholesterol compared with VLDL fractions (ie, the cholesterol to TG ratios were always greater for IDL and RLP v VLDL, $P < .001$).

The relationships between apoC-III parameters and TRL remnant lipoprotein levels were determined by linear regression (Table 5). All 4 remnant parameters were correlated with each other. Total plasma apoC-III was related to all remnant

Table 4. Plasma Remnant Lipoprotein Parameters in NL and HLP Subjects

Parameter	NL (n = 10)	HLP		
		Type IIa (n = 10)	Type IIb (n = 10)	Type IV (n = 10)
VLDL-C	0.21 ± 0.06	0.17 ± 0.04	1.25 ± 0.18‡	1.39 ± 0.18¶
VLDL-TG	0.75 ± 0.14	0.80 ± 0.12	3.23 ± 0.39‡	3.56 ± 0.61¶
VLDL-C/VLDL-TG	0.25 ± 0.06	0.21 ± 0.04	0.38 ± 0.04†	0.39 ± 0.04§
VLDL-C/plasma TG	0.15 ± 0.04	0.14 ± 0.03	0.31 ± 0.03‡	0.36 ± 0.03‖
IDL-C	0.15 ± 0.04	0.18 ± 0.04	0.48 ± 0.08†	0.35 ± 0.08§
IDL-TG	0.19 ± 0.03	0.16 ± 0.02	0.24 ± 0.03	0.18 ± 0.04
IDL-C/IDL-TG	0.84 ± 0.19	1.19 ± 0.22	1.93 ± 0.21*	1.88 ± 0.26¶
ISL apoE	1.31 ± 0.15	1.52 ± 0.11	3.26 ± 0.26‡	2.52 ± 0.20¶
RLP-C	0.22 ± 0.01	0.21 ± 0.01	0.50 ± 0.07‡	0.58 ± 0.11¶
RLP-TG	0.24 ± 0.03	0.18 ± 0.02	0.97 ± 0.18‡	1.12 ± 0.32§
RLP-C/RLP-TG	1.09 ± 0.17	1.31 ± 0.16	0.57 ± 0.05‡	0.59 ± 0.05¶

NOTE. Values represent the mean ± SE and are expressed in mmol/L.

* $P < .05$, †$P < .01$, ‡$P < .001$ v type IIa by unpaired t test.

§ $P < .05$, ‖$P < .01$, ¶$P < .001$ v NL by unpaired t test.

Table 5. Correlation Between Plasma Remnant Lipoprotein Parameters and ApoC-III Concentrations in Total Plasma and Lipoprotein Fractions

Parameter	VLDL-C	IDL-C	ISL ApoE	RLP-C
Total apoC-III	.850†	.500*	.517†	.637†
VLDL apoC-III	.919†	.441*	.534†	.782†
IDL/LDL apoC-III	—	—	—	—
HDL_{prec} apoC-III	—	—	—	—
LpB_{prec} apoC-III	.895†	.467*	.534†	.716†
Ratio $apoC\text{-}III_{prec}$	−.533†	—	−.569†	−.480*
TRL apoC-III	.898†	—	.420*	.792†
ISL apoC-III	.740†	.604†	.689†	.450*
HDL_{fplc} apoC-III	—	—	—	—
LpB_{fplc} apoC-III	.904†	.459*	.536†	.729†
Ratio $apoC\text{-}III_{fplc}$	−.677†	—	−.685†	−.608†

NOTE. Values represent statistically significant correlations (*r*) for all subjects combined (N = 40). Correlation coefficients that are not significant are not shown.

* $P < .01$.

† $P < .001$.

parameters. VLDL, TRL, LpB_{prep}, and LpB_{fplc} apoC-III were also correlated with every remnant parameter. The strongest correlations were with VLDL-C ($r = .919$, $r = .898$, $r = .895$, and $r = .904$, $P < .001$ for VLDL, TRL, LpB_{prep}, and LpB_{fplc} apoC-III, respectively), closely followed by RLP-C ($r = .782$, $r = .792$, $r = .716$, and $r = .729$, $P < .001$ for VLDL, TRL, LpB_{prep}, and LpB_{fplc} apoC-III, respectively). In contrast to IDL/LDL apoC-III, ISL apoC-III levels were significantly related to all remnant parameters. Interestingly, there was no relationship between HDL apoC-III (isolated by UTC/precipitation or gel filtration) and remnant parameters. The apoC-III ratios were negatively correlated ($P < .001$) with VLDL-C, ISL apoE, and RLP-C, but not with IDL-C.

DISCUSSION

The results of the present study demonstrate that HTG, due to an elevation of total plasma TG and VLDL-TG, is associated with a significant increase in the plasma apoC-III concentration, due to increased amounts of apoC-III associated with TRL and their remnants. HC, on the other hand, resulting from an elevation of LDL-C, had little effect on these parameters (Tables 2 and 3). Recent data from the ECTIM Study (Etude Cas-Temoins sur l'Infarctus du Myocarde) involving French and Irish subjects (N = 489) without coronary heart disease have also demonstrated that total plasma apoC-III and apoC-III-LpB are largely independent of plasma LDL levels.[27] Together, these results are consistent with the concept that the plasma apoC-III concentration and distribution are strongly linked to the TG status of human plasma.[7-9] Increased levels of TRL favor the exchange of apoC-III from HDL to TRL, and at the same time, limited TRL lipolysis reduces the transfer of apoC-III from TRL to HDL.[10] HTG is also associated with increased hepatic production of VLDL apoC-III.[28] The net result is that apoC-III accumulates in the TRL fraction. The negligible effect of LDL on plasma and lipoprotein apoC-III levels suggests that circulating LDL have little influence on plasma apoC-III metabolism, and conversely (despite the well-documented ability of apoC-III to inhibit the binding of LDL to the LDL receptor[29,30]), apoC-III has little influence on the rate or extent of plasma LDL catabolism. This is consistent with results from transgenic mice overexpressing human apoC-III, whereby pronounced HTG is associated with a significant increase in plasma TRL but relatively little change in the plasma concentration of LDL.[31,32] We can therefore conclude that relative or absolute changes in apoC-III levels in humans are a clear reflection of changes in plasma TRL metabolism and are not indicative of perturbed LDL metabolism.

The second question addressed in this study is the extent to which elevated levels of TG and LDL can affect plasma concentrations of remnant lipoproteins. In a previous study involving the same group of individuals, we demonstrated that increased levels of remnant lipoproteins isolated with an immunoaffinity gel (ie, RLP) were characteristic of patients with increased levels of TG and not increased levels of LDL.[33] However, TRL remnants are difficult to isolate, and no single biochemical technique can accurately account for these particles' large range of size, density, and/or composition.[20] For this reason, in the present study, we have compared 4 parameters reflecting remnant lipoprotein levels: (1) cholesterol enrichment of ultracentrifugally isolated VLDL, (2) cholesterol and TG concentration of plasma IDL ($1.006 < d < 1.019$ g/mL), (3) plasma concentration of apoE in ISL, and (4) cholesterol and TG concentration of plasma RLP (Table 4). All 4 parameters were consistently found to be higher (2- to 5-fold) in type IV compared with NL subjects and in type IIb compared with type IIa patients. Remnant levels were not different in type IIa versus NL nor in type IIb versus type IV patients—these groups having been matched for total plasma TG concentration. We can therefore conclude that increased remnant lipoprotein levels are associated with increased levels of TRL and not LDL. Support for this conclusion is provided by the observations of Phillips et al,[34] showing that IDL-C concentrations were more strongly correlated with plasma TG and VLDL-C concentrations than with LDL-C or apoB levels in 335 men and women participating in a coronary angiography study. The lack of a strong association between plasma levels of IDL and LDL is also supported by the results of Mabuchi et al[35] showing that heterozygotic and homozygotic familial hypercholesterolemic patients with significantly different LDL-C levels of 254 ± 59 and 432 ± 66 mg/dL, respectively, had similar IDL-C levels (26 ± 11 *v* 31 ± 12 mg/dL). Plasma remnant accumulation in the fasted state (as evidenced by the presence of double prebeta lipoproteinemia) has also been shown to be more prevalent in patients with CHL or HTG versus patients with HC alone.[36] Furthermore, chylomicron remnant accumulation in the fed state, as assessed by measuring the plasma retinyl ester concentration after ingestion of vitamin A, is characteristic of HTG type III or type IV HLP patients, rather than HC patients with type IIa HLP.[37] Data from a number of studies therefore confirm that remnant lipoprotein accumulation in plasma is a characteristic of HTG patients, rather than patients with increased levels of LDL.

The third and most important question addressed by the present study is the extent to which plasma and lipoprotein

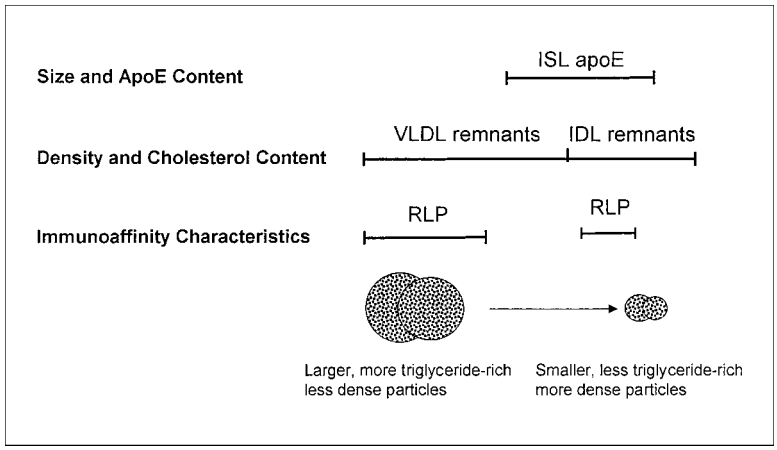

Fig 1. Diagrammatic representation of the relationship between different remnant lipoprotein parameters and the way in which they cover the full spectrum of remnant lipoproteins in the circulation. ISL apoE is apoE in ISL isolated by FPLC; VLDL and IDL remnants are lipoproteins in the d < 1.006 g/mL and 1.006 < d < 1.019 g/mL ultracentrifugal fractions, respectively. RLP were isolated by immunoaffinity gel.

levels of apoC-III reflect the accumulation in plasma of TRL remnants. By assessing 4 different remnant lipoprotein parameters, our results have taken into consideration the fact that not all remnant lipoproteins are identical. Some are large and more TG-enriched (isolated as VLDL), some are metabolic intermediates (characterized by their intermediate size and presence of apoE, ie, ISL apoE), and some are smaller cholesterol-enriched remnants (isolated as IDL). It is impossible to ascertain exactly how these parameters cover the full spectrum of remnant lipoproteins in circulating blood. However, we hypothesize that their relationship is as depicted in Fig 1. Irrespective of the exact relationship between these different remnant parameters, we found that they were all significantly correlated with total, VLDL, ISL, LpB_{prec}, and LpB_{fplc} apoC-III concentrations (Table 5). As expected, the apoC-III ratios, ie, HDL apoC-III/LpB apoC-III (identified previously to be an index of TRL lipolysis[12]), were negatively correlated with remnant parameters (except IDL-C). Stronger correlations tended to exist between apoC-III parameters and cholesterol in VLDL and RLP than with IDL-C and ISL apoE, indicating that apoC-III was associated to a greater extent with larger, less dense TRL remnants. This is contrary to the view expressed by Blankenhorn et al[16] and Hodis et al[17] that apoC-III in VLDL is associated with smaller, denser, more atherogenic VLDL. Their opinion is based on studies by Carlson and Ballantyne[38] and Kane et al[39] showing that smaller and more dense TRL have a higher apoC-III to apoC-II ratio than larger TRL. However, apoC-III enrichment of smaller particles relative to apoC-II is more a function of decreased amounts of apoC-II than increased amounts of apoC-III. Relative to apoB, larger particles always have more apoC-III than smaller particles.[39] In addition, apoC-III levels are more strongly correlated with total TG and VLDL-TG (Table 3) than with remnant levels (Table 5), providing additional evidence that apoC-III is a characteristic of larger rather than smaller TRL. The strength of the correlation between apoC-III and TG levels in both total plasma and VLDL is so strong, it appears that apoC-III is a "chaperone" of TG molecules in the circulation. This indicates that apoC-III parameters are more indicative of the balance between TRL TG production and lipolysis than of the extent of TRL remnant accumulation.

A final point worth considering is that even though LpB apoC-III levels were positively correlated with TG and TRL remnant parameters and apoC-III ratios were negatively correlated, these associations were due to significant changes in TRL and LpB apoC-III levels and not changes in HDL apoC-III levels. Patients in the present study did not have significantly different levels of HDL apoC-III using either separation method (Table 2), and HDL apoC-III levels were not significantly correlated with either TG or remnant parameters (Tables 3 and 5). Similarly, in the ECTIM study, HDL apoC-III levels (apoC-III-Lp non-B) were not negatively correlated with plasma TG status.[27] These data suggest that the levels of apoC-III in VLDL, TRL, or LpB (or apoC-III ratios) are appropriate indicators of plasma TRL metabolism, but by itself, the HDL apoC-III level is not. Although this is contrary to currently held belief,[12,13,16,17] several studies have shown that a significant proportion (30% to 60%) of total plasma apoC-III is not able to transfer between TRL and HDL[28,40,41] and also that HDL apoC-III can be produced in vivo independently of VLDL apoC-III.[28] Previous studies showing significant associations between HDL apoC-III levels and the progression of coronary artery atherosclerosis[16] need to be interpreted in this light.

In conclusion, the present data demonstrate that (1) increased amounts of apoC-III associated with plasma VLDL, TRL, or apoB-containing lipoproteins, as well as increased levels of TRL remnant lipoproteins, are a characteristic of HTG patients rather than patients with increased levels of LDL, and (2) plasma levels of apoC-III in VLDL, TRL, or LpB, as well as HDL apoC-III to LpB apoC-III ratios, are strongly correlated with circulating levels of TRL, although these apoC-III parameters more closely reflect the balance between TRL TG production and lipolysis than the extent of plasma TRL remnant accumulation.

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