Apolipoprotein C-III isoforms: kinetics and relative implication in lipid metabolism

Jean-François Mauger,[*] Patrick Couture,[†] Nathalie Bergeron,[§] and Benoît Lamarche,[1,*]

Institute on Nutraceuticals and Functional Foods,[*] and Department of Food Sciences and Nutrition,[§] Laval University, Québec, Canada; and the Lipid Research Center,[†] Laval University Medical Center, Québec, Canada

Abstract **Apolipoprotein C-III (apoC-III) production rate (PR) is strongly correlated with plasma triglyceride (TG) levels. ApoC-III exists in three different isoforms, according to the sialylation degree of the protein. We investigated the kinetics and respective role of each apoC-III isoform in modulating intravascular lipid/lipoprotein metabolism. ApoC-III kinetics were measured in a sample of 18 healthy men [mean age (±SD) 42.1 ± 9.5 years, body mass index 29.8 ± 4.6 kg/m^2] using a primed-constant infusion of L-(5,5,5-D3) leucine for 12 h. Mono-sialylated and di-sialylated apoC-III (apo-CIII$_1$ and apoC-III$_2$) exhibited similar PRs (means ± SD, 1.22 ± 0.49 mg/kg/day vs. 1.15 ± 0.59 mg/kg/day, respectively) and similar fractional catabolic rates (FCRs) (0.51 ± 0.13 pool/day vs. 0.61 ± 0.24 pool/day, respectively). Nonsialylated apoC-III (apoC-III$_0$) had an 80% lower PR (0.25 ± 0.12 mg/kg/day) and a 60% lower FCR (0.21 ± 0.07 pool/day) ($P < 0.0001$ for comparison with CIII$_1$ and CIII$_2$ isoforms). The PRs of apoC-III$_1$ and apoC-III$_2$ were more strongly correlated with plasma TG levels ($r > 0.8$, $P < 0.0001$) than was apoC-III$_0$ PR ($r = 0.54$, $P < 0.05$). Finally, the PR of apoC-III$_2$ was strongly correlated with the proportion of LDL <255 Å ($r = 0.72$, $P = 0.002$). These results suggest that all apoC-III isoforms, especially the predominant CIII$_1$ and CIII$_2$ isoforms, contribute to hypertriglyceridemia and that apoC-III$_2$ may play a significant role in the expression of the small, dense LDL phenotype.**—Mauger J-F., P. Couture, N. Bergeron, and B. Lamarche. **Apolipoprotein C-III isoforms: kinetics and relative implication in lipid metabolism.** ***J. Lipid Res.*** **2006.** 47: **1212–1218.**

Supplementary key words VLDL metabolism • metabolic syndrome • triglycerides • LDL particle size • sialylation

Emphasis is continuously put on plasma triglyceride (TG) levels as a key correlate of many features of the metabolic syndrome, such as abdominal obesity, type 2 diabetes, lower levels of anti-atherogenic HDL-cholesterol (HDL-C), and higher levels of triglyceride-rich lipoproteins (TRLs) (1–3). Increased concentrations of plasma triglycerides are also thought to promote the expression of the type B LDL phenotype, characterized by the predominant presence of more-atherogenic, small, dense LDL particles (4, 5). In that context, there is a renewed interest in better understanding the mechanisms modulating plasma TG levels.

Apolipoprotein C-III (apoC-III) is a protein secreted mostly by the liver and, to a lesser extent, by the intestine (3). In circulation, it is associated with both TRLs and HDL (6). ApoC-III is present in three isoforms that are termed apoC-III$_0$, apoC-III$_1$, and apoC-III$_2$, depending on the number of sialic acid molecules (0 to 2) terminating the oligosaccharidic portions of the protein (7). Each isoform has been shown to contribute, respectively, to approximately 10, 55, and 35% of the total apoC-III levels in circulation (8). Total plasma apoC-III levels have been identified as a major determinant of triglyceridemia (9). Proposed mechanisms underlying the hypertriglyceridemic effect of apoC-III comprise inhibition of lipoprotein lipase (LPL) activity, disruption of the interaction of TRLs with vessel wall heparan sulfate proteoglycans, and lower clearance of apoB-containing lipoproteins by LDL and LDL-related receptors (10–12).

The human in vivo kinetics of apoC-III has recently been studied in both healthy and hypertriglyceridemic individuals, using stable isotopes. These studies have been relatively consistent in revealing that plasma apoC-III pool size (PS) and production rate (PR), rather than plasma apoC-III fractional catabolic rate (FCR), were the major determinants of plasma TG levels (13). Other studies have also indicated that apoC-III concentrations and PR were strongly and positively associated with increased production rate of VLDL-TG (14). It must be stressed that these previous studies have only investigated the kinetics of the predominant apoC-III isoform, apoC-III$_1$. To the best of

Manuscript received 18 October 2005 and in revised form 1 February 2006.

Published, JLR Papers in Press, February 22, 2006.
DOI 10.1194/jlr.M500455-JLR200

Abbreviations: apoC-III, apolipoprotein C-III; BMI, body mass index; FCR, fractional catabolic rate; HDL-C, HDL-cholesterol; LPL, lipoprotein lipase; PR, production rate; PS, pool size; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

[1] To whom correspondence should be addressed.
e-mail: benoit.lamarche@inaf.ulaval.ca

our knowledge, no study to date has investigated the contribution of the other C-III isoforms to triglyceridemia and other dyslipidemic features of the metabolic syndrome, despite their significant contribution to the total apoC-III pool size. Furthermore, despite the extensively described strong negative relationship between TG levels and LDL particle size, no study yet has examined the impact of apoC-III isoform kinetics and distribution on LDL particle size.

The purpose of the present study was to compare the kinetics of each apoC-III isoform in order to determine their respective implications in lipid/lipoprotein metabolism and to evaluate their contribution to the expression of the small, dense LDL phenotype.

METHODS

Subjects

Eighteen Caucasian men aged 42.1 ± 9.5 years with a mean body mass index (BMI) of 29.8 ± 4.6 kg/m^2, nonsmokers, normoglycemic, normolipidemic, and free of any thyroid, endocrine, cardiovascular, hepatic, or renal disorders were recruited in the Québec City area. Subjects who experienced significant gain or loss of weight (>3 kg) in the 2 months preceding the study, who had excessive alcohol intake (>30 g/day), had taken drugs, or had unusual dietary habits were excluded. Macronutrient intake, as assessed by a 3 day food record obtained before the study (2 week days and 1 weekend day), was comparable between subjects (data not shown). All subjects gave informed consent, and the study protocol was accepted by the Clinical Research Ethical Committee of Laval University.

Lipid profile

Plasma and HDL lipid concentrations were measured by enzymatic methods on a Technicon RA-500 analyzer (Bayer Corp.; Tarrytown, NY) as previously described (15). Plasma VLDL ($\rho < 1.006$ g/ml) was isolated by ultracentrifugation, and the HDL fraction was obtained after precipitation of LDL in the infranatant ($\rho > 1.006$ g/ml) with heparin and $MnCl_2$. The cholesterol, TG, and phospholipid content of the infranatant fraction were measured before and after the precipitation step. Plasma apoA-I and VLDL-apoB-100 concentrations were measured by nephelometry (16), and apoB-100 concentrations of IDL and LDL fractions were obtained by commercially available enzyme-linked immunosorbent assay (CardioCHEK; AlerCHEK, Portland, ME).

Kinetic studies

Kinetics of apoC-III were determined using a primed-constant infusion of L-(5, 5, 5,-D3)-leucine carried out for 12 hours as described previously (17). Subjects were studied in the constantly fed state to mimic the postprandial state. Every 30 min during the 3 hours that preceded the infusion, as well as during the 12 hour infusion (total of 15 hours), subjects received a meal consisting of 1/30th of their daily caloric intake. The bolus dose and injection rate of [5, 5, 5,-2H3] L-leucine were calculated on the basis of the subject's weight (10 μmol/kg and 10 μmol/kg/hour, respectively). Blood samples (20 ml) were collected via a second intravenous line into vacutainer tubes containing EDTA (to a final concentration of 0.1%) at times 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours.

ApoC-III isoform isotopic enrichment determination

ApoC-III was isolated from the $\rho < 1.25$ g/ml plasma fraction obtained after centrifugation of whole plasma for 48 hours at 50,000 rpm at 4°C in a Beckman 50.4 Ti rotor. The infranatant was then dialysed overnight in an NaCl-Tris-base EDTA buffer, incubated with cysteamine for 4 hours at 37°C, and delipidated using acetone-ethanol and diethylic ether as described previously (18). All apolipoproteins were then separated using preparative isoelectric focusing on polyacrylamide-urea gels (pH gradient 4–7). Protein bands were revealed with Coumassie blue, and gels were scanned and analyzed using Imagemaster 1-D Prime computer software (Amersham Pharmacia Biotech). The proportion of the area under the curve associated with each of the isoforms on the densitometric scan was used to derive the specific isoform concentrations as described previously (19). After analysis, bands were excised and hydrolyzed in 6 N HCl at 110°C for 24 hours. Free amino acids in the hydrolysate were then derivatized by treatment with trifluoroacetic acid and trifluoroacetic acid anhydride at 110°C (20). The derivatized amino acids were extracted into benzene and analyzed on a gas chromatograph/mass spectrometer (GC 6890N, MS 5973N; Agilent Technologies, Palo Alto, CA). Amino acids were ionized by methane-negative chemical ionization, and selective ion monitoring at *m/z* 212 and 209 was used to determine the isotopic ratio (deuterated/non-deuterated leucine) for each sample. Tracer-to-tracee ratios were calculated from the isotopic ratio using the formula described previously (21).

ApoB-100 isotopic enrichment determination

The VLDL ($\rho < 1.006$ g/ml, 18 hours at 312,000 *g*) and IDL ($\rho = 1.006$–1.019 g/ml, 18 hours at 312,000 *g*) fractions were isolated from fresh plasma by sequential ultracentrifugation and analyzed as described previously (21).

Kinetic analysis

The FCR of apoA-I, apoB-100, and each apoC-III isoform was determined by fitting the tracer-to-tracee ratio to a monoexponential function using the SAAMII Program software (University of Washington, Department of Bioengineering, Seattle, WA). Data were fitted to the mathematical function $Z(t) = Z_p(1-e^{-k(t-d)})$, where Z(t) is the tracer-to-tracee ratio at time t, Z_p is the tracer-to-tracee ratio corresponding to the plateau of the curve representing the precursor amino acid pool, d is the delay time in h, and k is the FSR in pools per h. The enrichment plateau of VLDL apoB-100 with deuterium-labeled leucine was used as the forcing function for precursor pool enrichment. Absolute PR was calculated (in mg/kg/d) using the formula:

$$PR = \frac{\text{FSR (pools/day)} \times \text{plasma apolipoprotein pool size (mg)}}{\text{body weight (kg)}}$$

Pool sizes were calculated as the plasma concentration of each apoC-III (mg/l) multiplied by estimated plasma volume (value fixed at 0.045 l/kg body weight).

LDL particle size characterization

LDL size phenotype was characterized using nondenaturing polyacrylamide gradient gel electrophoresis of whole plasma along with standards of known diameter as previously described (22).

Statistical analysis

Data were analyzed using SAS (version 8.2; SAS Institute, Inc., Cary, NC). Correlations were examined using Spearman rank

correlations, and the Student paired *t*-test procedure was used for comparison of the kinetic parameters of the different ApoC-III isoforms.

RESULTS

Study subjects

The physical characteristics and plasma lipid/lipoprotein profiles of the study subjects are presented in **Table 1**. Participants were aged 42.1 ± 9.5 years, and most of them (15/18) were either overweight (BMI >25 kg/m^2, 7 subjects) or obese (BMI >30 kg/m^2, 8 subjects). As a group, study subjects had relatively normal plasma triglyceride and HDL-C levels (1.67 ± 0.97 mmol/l and 1.13 ± 0.23 mmol/l, respectively) and near-normal total and LDL cholesterol levels (5.05 ± 1.15 mmol/l and 3.28 ± 0.97 mmol/l, respectively).

Concentrations and proportions of apolipoprotein C-III isoforms

Mean fasting (±SD) concentrations of apoC-III$_0$, apoC-III$_1$, and apoC-III$_2$ were 26.1 ± 10.2 mg/l, 54.0 ± 20.7 mg/l, and 39.9 ± 17.2 mg/l, respectively ($P < 0.0001$ for comparison between isoforms), reflecting respective mean proportions of 22.0 ± 2.0%, 45.0 ± 2.9%, and 33.0 ± 3.7% of total apoC-III ($P < 0.0001$ for comparison between isoforms). No relationship was observed between anthropometric variables such as body mass index, waist circumference and weight, and inter-individual variations in the proportion of apoC-III isoforms. Significant positive correlations were observed between BMI, visceral adiposity measured by standardized axial tomography as described previously (23), and plasma concentrations of apoC-III$_1$ ($r = 0.59$, $P = 0.01$ and $r = 0.57$, $P = 0.02$, respectively) but not with the other two isoforms.

Kinetics of apolipoprotein C-III isoforms

Enrichment curves of the three apoC-III isoforms in deuterated leucine over time are depicted in **Fig. 1**. Nonsialylated ApoC-III (ApoC-III$_0$) was characterized by a slower enrichment rate compared with both apoC-III$_1$ and apoC-III$_2$ isoforms, which exhibited relatively similar enrichment rates. **Table 2** shows the calculated kinetic parameters for the different apoC-III isoforms. ApoC-III$_0$ was the isoform with the lowest PR and FCR ($P > 0.0001$, compared with the other two isoforms), whereas apoC-III$_1$ and apoC-III$_2$ exhibited relatively similar kinetics.

TABLE 1. Characteristics of study subjects

Variables	Mean ± SD
N	18
Age (years)	42.1 ± 9.5
Weight (kg)	90.1 ± 16.5
BMI (kg/m^2)	29.8 ± 4.6
Triglycerides (mmol/l)	1.67 ± 0.97
Total cholesterol (mmol/l)	5.05 ± 1.15
LDL cholesterol (mmol/l)	3.28 ± 0.97
HDL cholesterol (mmol/l)	1.13 ± 0.23

BMI, body mass index.

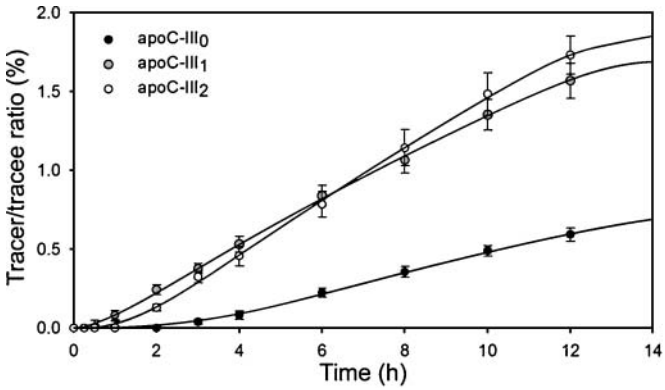

Fig. 1. Modeled enrichment curves of the three apoC-III isoforms with deuterated leucine during the 12 hour constant infusion. The circles represent average tracer-to-tracee ratio for each given time point, and the curves fit the values predicted by the model.

ApoC-III isoforms and plasma lipids and lipoproteins

Correlations between apoC-III isoform kinetics and plasma lipids are shown in **Table 3**. The PSs and PRs of all apoC-III isoforms, but not the FCRs, were highly and positively correlated with VLDL triglycerides and VLDL-cholesterol levels. HDL-cholesterol levels were inversely associated with all apoC-III isoform PSs and PRs.

The relationship between apoC-III isoform kinetics and apoB-100 and apoA-I kinetics was also investigated. Although the PSs of the three apoC-III isoforms were strongly and inversely correlated with VLDL apoB-100 FCR, only the PSs and concentrations of apoC-III$_0$ and apoC-III$_1$ showed significant positive relationships with the FCR of IDL apoB-100. The PSs of all apoC-III isoforms were strongly correlated with the apoB-100 PSs of all apoB-100-containing fractions, including LDL [LDL apoB-100 PS vs. apoC-III$_0$ PS: $r = 0.58$ ($P = 0.0187$), vs. apoC-III$_1$ PS: $r = 0.63$ ($P = 0.009$), vs. apoC-III$_2$ PS: $r = 0.64$ ($P = 0.0082$)]. There was no relationship between apoC-III kinetics and apoA-I FCR or PR, but a surprising positive association was found between all apoC-III isoform PSs and apoA-I PS.

ApoC-III isoforms and LDL particle size

The relationship between apoC-III isoform kinetics and LDL particle size was also investigated. Significant negative correlations were observed between the three apoC-III isoform PSs and LDL peak particle sizes (data not shown), but only the concentrations of apoC-III$_2$ were significantly associated with LDL peak particle size ($r = -0.57$, $P = 0.02$) and the proportion of LDL <255 Å ($r = 0.56$, $P = 0.02$). Furthermore, as depicted in **Fig. 2**, only the apoC-III$_2$ PR showed a significant association with LDL peak particle size. This relationship remained highly significant when adjusted for plasma TG levels ($P = 0.011$).

TABLE 2. Plasma kinetics of each apoC-III isoform in the constantly fed state

Isoform	Concentration	Pool Size	FCR	PR
	mg/l	*mg*	*Pools/day*	*mg/kg/day*
apoC-III$_0$	26.1 ± 10.2^a	107.4 ± 49.9^a	0.215 ± 0.068^a	0.250 ± 0.121^a
apoC-III$_1$	54.0 ± 20.7^b	223.7 ± 105.7^b	0.509 ± 0.129^b	1.222 ± 0.487^b
apoC-III$_2$	39.9 ± 17.2^c	164.7 ± 85.2^c	0.615 ± 0.236^b	1.150 ± 0.587^b

FCR, fractional catabolic rate; PR, production rate. Values with different superscripts are statistically different ($P < 0.0001$).

DISCUSSION

All recent studies that have investigated apoC-III kinetics using stable isotopes have limited their analysis to the predominant mono-sialylated isoform of the peptide, and only a few studies, performed with radioisotopes (24–26), have considered both the mono-sialylated and di-sialylated isoforms. To the best of our knowledge, no study has yet compared the association between each of the three apoC-III isoforms and circulating lipid levels and apolipoprotein B-100 and A-I kinetics. Consequently, the purpose of the present study was to examine the extent to which each apoC-III isoform correlates with variations in lipoprotein/lipid metabolism. Also, because LDL particles are the end product of the lipoprotein hydrolysis pathway and because their size is tightly correlated with triglyceridemia, we also investigated the relationship between apoC-III isoform kinetics and LDL electrophoretic characteristics.

In the present study, non-, mono-, and di-sialylated apoC-IIIs, respectively, accounted for approximately 22, 45, and 33% of the total circulating apoC-III pool, which is concordant with previous data reporting isoform relative proportions of 15, 55, and 35%, respectively (27). Although BMI and visceral fat correlated with plasma apoC-III$_1$ concentrations, no correlation was observed between the relative proportion of each isoform and anthropometric variables such as visceral adipose tissue, weight, and BMI. This suggests that the degree of sialylation of apoC-III may not be related to plasma lipid levels or obesity indices, or vice-versa.

We observed that the less-predominant isoform of apoC-III, apoC-III$_0$, had a significantly slower PR and FCR compared with the predominant apoC-III$_1$ and apoC-III$_2$ isoforms. Studies performed by Huff et al. (24) in the early 1980s using radioisotopes suggested that the mono- and di-sialylated isoforms of apoC-III had similar clearance rates from the circulation (0.648 pool/day and 0.624 pool/day, respectively). The FCR values of apoC-III$_1$ and apoC-III$_2$ measured using stable isotopes in the present experiments were in the same range as those obtained using radioisotopes (0.509 pool/day and 0.615 pool/day, respectively). In our study, the difference between apoC-III$_1$ and apoC-III$_2$ FCRs was almost statistically significant (*t*-test, $P = 0.14$). This suggests possible subtle differences in underlying mechanisms of clearance, which contradicts the hypothesis of a common clearance pathway for both isoforms, as proposed by Huff et al. (24).

Clearance pathways for apoC-III are largely unknown. No correlation was found between apoC-III isoform FCR and the FCR of apoB-100 in VLDL and IDL. This result, along with the observation that the FCR of apoB-100 in VLDL (8.5 ± 5.4 pools/day) and IDL (6.6 ± 4.6 pools/day) are more than 10 times greater than the highest FCR value among apoC-III isoforms (apoC-III$_2$, 0.615 ± 0.236 pool/day), suggests that the intravascular catabolism of apoC-III may be independent of apoB-100. On the other hand, a correlation trend was observed between apoC-III$_0$

TABLE 3. Spearman correlation coefficient between apoC-III isoform kinetics and apolipoprotein metabolism and lipid levels

	VLDL		VLDL apoB-100			IDL		IDL apoB-100			HDL		HDL apoA-I		
	TG	Chol	PS	PR	FCR	TG	Chol	PS	PR	FCR	TG	Chol	PS	PR	FCR
PS															
apoC-III$_0$	0.83^d	0.82^d	0.86^d	0.06	−0.56^a	0.27	−0.02	0.66^b	−0.05	−0.57^a	0.06	−0.58^b	0.51^a	−0.15	0.08
apoC-III$_1$	0.84^d	0.81^d	0.88^d	0.04	−0.62^b	0.22	−0.07	0.71^c	−0.05	−0.63^b	0.08	−0.51^a	0.58^b	−0.17	0.01
apoC-III$_2$	0.87^d	0.89^d	0.82^d	0.18	−0.54^a	0.11	−0.04	0.77^c	0.10	−0.42	0.27	−0.50^a	0.53^a	−0.09	−0.04
PR															
apoC-III$_0$	0.54^a	0.53	0.47^a	0.32	−0.06	0.16	−0.09	0.18	−0.11	−0.14	−0.01	−0.55^a	0.00	0.28	0.51
apoC-III$_1$	0.82^d	0.80^d	0.80^d	0.25	−0.44	0.31	0.09	0.61	0.00	−0.56^a	0.07	−0.65^b	0.32	0.01	0.21
apoC-III$_2$	0.76^c	0.72^c	0.61^b	0.35	−0.23	−0.06	−0.08	0.50	0.16	−0.25	0.40	−0.56^a	0.21	0.36	0.34
FCR															
apoC-III$_0$	0.05	0.10	0.05	0.12	0.07	0.00	−0.09	−0.11	−0.24	0.08	−0.18	−0.31	−0.12	0.27	0.45
apoC-III$_1$	0.04	0.07	0.03	0.07	0.03	0.27	−0.03	−0.16	−0.26	−0.01	−0.30	−0.38	−0.26	0.13	0.42
apoC-III$_2$	0.15	0.25	0.31	0.12	−0.26	−0.20	−0.35	0.21	−0.05	−0.16	0.21	−0.38	−0.03	0.14	0.34

ApoC, apolipoprotein C; Chol, cholesterol; PS, pool size; TG, triglyceride.
$^a P < 0.05$.
$^b P < 0.01$.
$^c P < 0.001$.
$^d P < 0.0001$.

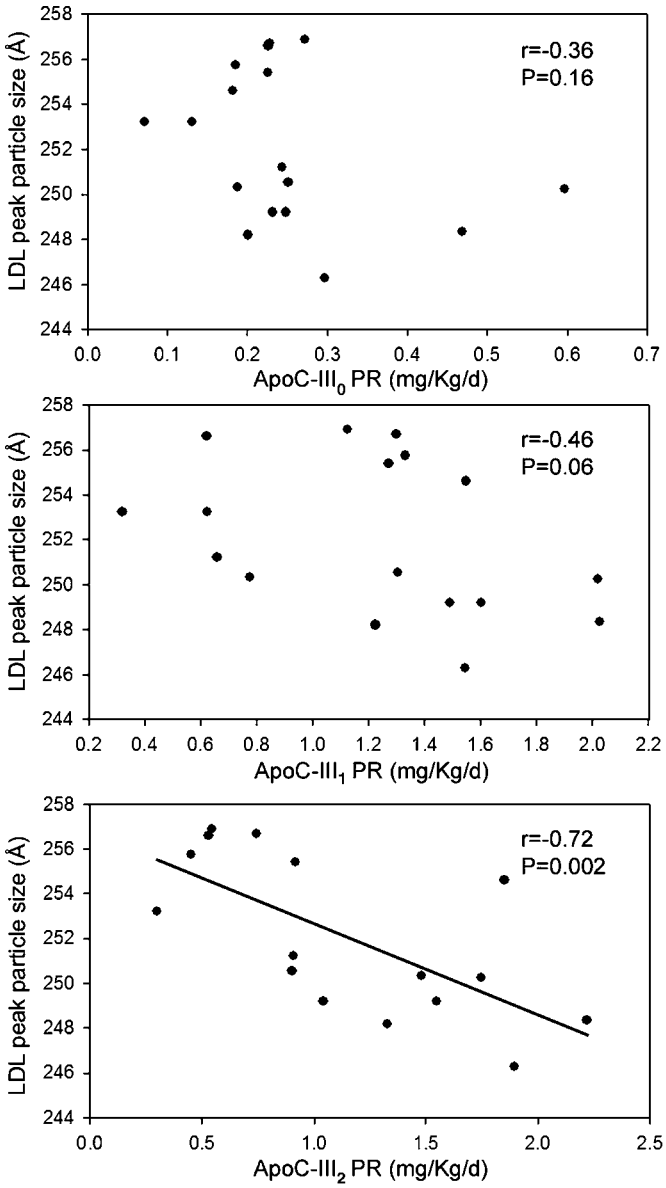

Fig. 2. Relation between the production rate (PR) of each apoC-III isoform and LDL peak particle size.

and apoC-III$_1$ FCRs and apoA-I FCR. This observation suggests that the non- and mono-sialylated isoforms of apoC-III may be preferentially cleared from the circulation through pathways that are shared with apoA-I, although this hypothesis remains to be formally tested.

The sialylation of proteins, and especially of apolipoproteins, is poorly understood. Apolipoprotein sialylation is an intracellular process driven by a family of Golgi – membrane-bound enzymes known as sialyltransferases (28). The proposed functions of sialylation include stabilization of protein conformation, resistance to protease, charge, protein targeting, and developmental regulation (29). Despite the fact that neuraminidase, the lysosomal enzyme responsible for the specific removal of sialic acid residues of sialylated proteins, has been detected in the circulation, it is thought that no desialylation occurs in the plasma because the optimal pH for the reaction ranges between 4 and 5 (30). It is therefore most likely that the low PR and FCR of apoC-III$_0$ compared with the two other isoforms may simply reflect the fact that only a limited amount of apoC-III avoids the sialylation process prior to its secretion in the circulation. This hypothesis would be concordant with data indicating that protein sialylation is a variable and nonobligatory step for further processing and secretion (30). On the other hand, the lower FCR of apoC-III$_0$ may be due to its relatively smaller pool size. Because apoC-III is known to exchange between lipoproteins, the smaller pool size of this isoform may reduce its probability of entering the catabolic pathways leading to its clearance compared with other more-prevalent isoforms. The lack of evidence for a metabolic relationship between the different isoforms of apoC-III in circulation was the main reason that we did not model their kinetics together.

Previous work on apoC-III kinetics has emphasized the strong relationship between total plasma apoC-III$_1$ PS as well as PR and total plasma and VLDL-TG levels (13, 31). The present study showed that this association was consistent for all three apoC-III isoforms. Indeed, the PSs and PRs of all apoC-III isoforms correlated positively with the PS of VLDL apoB-100 and negatively with the FCR of VLDL apoB-100. ApoC-III$_1$ PR and PS also correlated with the PS and PR of IDL apoB-100. The lack of association between apoC-III$_2$ and apoC-III$_0$ PR and IDL kinetics may be due to the smaller PS of these isoforms in relative terms, which in turn may limit their impact on remnant lipoprotein metabolism compared with apoC-III$_1$. These data suggest that all apoCIII isoforms may contribute to the inhibition of the LPL-mediated intravascular hydrolysis of TRL, resulting in a delayed catabolism of apoB-100 in the VLDL fraction, whereas only the predominant isoform, apoC-III$_1$, would be related to the metabolism of TRL remnants.

We also observed a significant positive correlation between the PSs of all apoC-III isoforms and plasma apoA-I PS. This was surprising, considering the strong relationships between apoC-III and plasma TG levels on the one hand and the inverse relationship between TG levels and HDL-C on the other hand. However, almost 50% of apoC-III has been shown to be HDL bound, and a significant positive relationship between HDL apoC-III PS and HDL apoA-I PS has previously been reported by Cohn et al. (32).

The strong correlation between plasma TG levels and LDL peak particle size has been extensively described (4, 5). Our data suggest that the degree of sialylation of apoC-III may be an important modulator of LDL particle size. Very few experiments have been undertaken to elucidate the impact of the apoC-III degree of sialylation on lipoprotein/lipid metabolism. The contribution of the sialic acid content of apoC-III to the in vitro activity of the purported lipolysis-stimulated receptor (LSR) of rat liver, which is thought to be involved in TRL clearance by hepatocytes, has been studied previously. The study showed that although apoC-III$_2$ has an apparent 2-fold greater affinity for VLDL than do the other two apoC-III isoforms,

its capacity to inhibit VLDL binding to LSR was almost 50% of that of the mono- and di-sialylated isoforms (33). It has also been demonstrated that hypertriglyceridemic subjects have a greater proportion of apoC-III in the di-sialylated isoform associated with VLDL (34). Another study reported that the apoC-III sialic acid content had no impact on its ability to inhibit the LPL-driven hydrolysis of lipoproteins (35). However, this later study only compared apoC-III_0 (neuraminidase-treated apoC-III_1) with apoC-III_1.

To the best of our knowledge, none of the previous in vitro studies examined per se the impact of VLDL enrichment with apoC-III_2 on LPL-driven hydrolysis. This leaves the possibility that VLDL with a higher content of apoC-III_2 may be less-readily hydrolyzed by LPL compared with VLDL with a normal apoC-III_2/apoC-III_1 ratio, as suggested previously (34). Therefore, to possibly explain the strong relationship between apoC-III_2 concentrations and PR and small, dense LDL particles, we hypothesize that apoC-III_2, because of its higher affinity for VLDL, may increase the VLDL apoC-III_2/apoC-III_1 ratio, thereby contributing to a greater inhibition of VLDL hydrolysis by LPL. This, in turn, would lead to a longer residence time of TRL and TRL remnants in the circulation and possibly to an increased cholesteryl ester transfer protein-mediated transfer of triglycerides to HDL and LDL in exchange for cholesterol. Ultimately, the TG content of LDL would most likely be hydrolyzed by hepatic triglyceride lipase to generate small, dense LDL particles. On the basis of this hypothesis, we would anticipate a stronger correlation between apoC-III_2 concentrations and VLDL-TG, compared to other apoC-III isoforms, which was not observed in the present study. We believe that this may be partly explained by the fact that only relatively normolipidemic subjects were investigated. Information on the proportion of each isoform associated within VLDL would also have been potentially useful for further investigation of this hypothesis. Investigating a more heterogeneous group of normo- and hypertriglyceridemic subjects and quantifying the proportion of apoC-III isoforms associated with HDL and VLDL should therefore be the focus of future studies.

Previous experiments have suggested that a proportion of apoC-III may not be available for free transfer, or equilibration, between HDL and TRL when transiting between the fasting and fed states and vice-versa (36, 37). To the best of our knowledge, the exchangeability of apoC-III in relation to its degree of sialylation has yet to be investigated. Because our study was performed using the total plasma lipoprotein fraction ($\rho < 1.25$ g/ml), it could not provide insightful information on the exchangeability of the different isoforms of apoC-III between HDL and TRL. However, it remains possible that the sialylation degree of the various apoC-III isoforms, by determining their affinity for specific lipoproteins, may also modulate their rate of exchange between lipoproteins. Therefore, it is possible that apoC-III_2 may exchange less rapidly from VLDL, but this, again, warrants further investigation. Finally, it has been reported that some apoC-III may possibly be lost when lipoproteins are subjected to ultracentrifugation. Cohn et al. (38) previously reported that the amount of apoC-III found in the bottom plasma fraction ($\rho > 1.21$ g/ml) after four sequential ultracentrifugation runs represented only 0.5% of total plasma apoC-III. On the basis of this finding, we assume that the single ultracentrifugation step in the present study was unlikely to have altered the relative proportions of each apoC-III isoform measured in the $\rho < 1.25$ g/ml fraction.

In conclusion, this study demonstrated that the kinetics of the two most predominant isoforms, apoC-III_2 and apoC-III_1, show the strongest associations with the expression of many features of the metabolic syndrome, such as hypertriglyceridemia and reduced VLDL and IDL apoB-100 catabolism. ApoC-III_2 concentrations and kinetics were also the strongest correlate of the expression of the small, dense LDL phenotype, despite the fact that apoC-III_2 is not the most predominant isoform of the protein in circulation. This suggests that apoC-III_2 may have the most deleterious impact on LDL particle size, which is an important risk factor for cardiovascular disease.

The authors would like to express grateful thanks to Mrs. Johanne Marin and Mr. George Cousineau for their valuable help and relevant advice. This study has been supported in part by an operating grant from the Canadian Institutes for Health Research. J-F.M. is a recipient of a studentship from the Canadian Institutes for Health Research. P.C. is a clinical scientist and is supported by the Fonds de la Recherche en Santé du Québec.

REFERENCES

1. Ruotolo, G., and B. V. Howard. 2002. Dyslipidemia of the metabolic syndrome. *Curr. Cardiol. Rep.* **4:** 494–500.
2. Chan, D. C., H. P. Barrett, and G. F. Watts. 2004. Dyslipidemia in visceral obesity: mechanisms, implications, and therapy. *Am. J. Cardiovasc. Drugs.* **4:** 227–246.
3. van Dijk, K. W., P. C. Rensen, P. J. Voshol, and L. M. Havekes. 2004. The role and mode of action of apolipoproteins CIII and AV: synergistic actors in triglyceride metabolism? *Curr. Opin. Lipidol.* **15:** 239–246.
4. Katzel, L. I., P. J. Coon, E. Rogus, R. M. Krauss, and A. P. Goldberg. 1995. Persistence of low HDL-C levels after weight reduction in older men with small LDL particles. *Arterioscler. Thromb. Vasc. Biol.* **15:** 299–305.
5. McNamara, J., J. Jenner, Z. Li, P. Wilson, and E. Schaefer. 1992. Change in LDL particle size is associated with change in plasma triglyceride concentration. *Arterioscler. Thromb.* **12:** 1284–1290.
6. Jong, M. C., M. H. Hofker, and L. M. Havekes. 1999. Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. *Arterioscler. Thromb. Vasc. Biol.* **19:** 472–484.
7. Ito, Y., J. L. Breslow, and B. T. Chait. 1989. Apolipoprotein C–III0 lacks carbohydrate residues: use of mass spectrometry to study apolipoprotein structure. *J. Lipid Res.* **30:** 1781–1787.
8. Juntti-Berggren, L., E. Refai, I. Appelskog, M. Andersson, G. Imreh, N. Dekki, S. Uhles, L. Yu, W. J. Griffiths, S. Zaitsev, et al. 2004. Apolipoprotein CIII promotes Ca2+-dependent beta cell death in type 1 diabetes. *Proc. Natl. Acad. Sci. USA.* **101:** 10090–10094.
9. Gerritsen, G., P. C. Rensen, K. E, Kypreos, V. I. Zannis, L. M. Havekes, and v. D. Willems. 2005. ApoC-III deficiency prevents hyperlipidemia induced by apoE overexpression. *J. Lipid Res.* **46:** 1466–1473.
10. Aalto-Setala, K., E. A. Fisher, X. Chen, T. Chajek-Shaul, T. Hayek, R. Zechner, A. Walsh, R. Ramakrishnan, H. N. Ginsberg, and J. L.

Breslow. 1992. Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apo CIII and reduced apo E on the particles. *J. Clin. Invest.* **90:** 1889–1900.
11. Ebara, T., R. Ramakrishnan, G. Steiner, and N. S. Shachter. 1997. 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.* **99:** 2672–2681.
12. Shachter, N. S. 2001. Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. *Curr. Opin. Lipidol.* **12:** 297–304.
13. Cohn, J. S., M. Tremblay, R. Batal, H. Jacques, C. Rodriguez, G. Steiner, O. Mamer, and J. Davignon. 2004. Increased apoC-III production is a characteristic feature of patients with hypertriglyceridemia. *Atherosclerosis.* **177:** 137–145.
14. Cohn, J. S., B. W. Patterson, K. D. Uffelman, J. Davignon, and G. Steiner. 2004. Rate of production of plasma and very-low-density lipoprotein (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male subjects with different body weights and levels of insulin sensitivity. *J. Clin. Endocrinol. Metab.* **89:** 3949–3955.
15. Archer, W. R., B. Lamarche, O. Deriaz, N. Landry, L. Corneau, J. P. Despres, J. Bergeron, P. Couture, and N. Bergeron. 2003. Variations in body composition and plasma lipids in response to a high-carbohydrate diet. *Obes. Res.* **11:** 978–986.
16. An, P., T. Rice, J. Gagnon, I. B. Borecki, J. Bergeron, J. P. Despres, A. S. Leon, J. S. Skinner, J. H. Wilmore, C. Bouchard, et al. 2000. Segregation analysis of apolipoproteins A-1 and B-100 measured before and after an exercise training program: the HERITAGE Family Study. *Arterioscler. Thromb. Vasc. Biol.* **20:** 807–814.
17. Desroches, S., M. E. Paradis, M. Perusse, W. R. Archer, J. Bergeron, P. Couture, N. Bergeron, and B. Lamarche. 2004. Apolipoprotein A-I, A-II, and VLDL-B-100 metabolism in men: comparison of a low-fat diet and a high-monounsaturated fatty acid diet. *J. Lipid Res.* **45:** 2331–2338.
18. Mauger, J. F., P. Couture, M. E. Paradis, and B. Lamarche. 2005. Comparison of the impact of atorvastatin and simvastatin on apoA-I kinetics in men. *Atherosclerosis.* **178:** 157–163.
19. Bugugnani, M. J., M. Koffigan, I. Kora, D. Ouvry, V. Clavey, and J. C. Fruchart. 1984. Rapid assessment of the distribution of apolipoproteins C-II and C-III subspecies in triglyceride-rich lipoproteins by isoelectric focusing. *Clin. Chem.* **30:** 349–351.
20. Dwyer, K. P., P. H. Barrett, D. Chan, J. I. Foo, G. F. Watts, and K. D. Croft. 2002. Oxazolinone derivative of leucine for GC-MS: a sensitive and robust method for stable isotope kinetic studies of lipoproteins. *J. Lipid Res.* **43:** 344–349.
21. Cobelli, C., G. Toffolo, and D. M. Foster. 1992. Tracer-to-tracee ratio for analysis of stable isotope tracer data: link with radioactive kinetic formalism. *Am. J. Physiol.* **262:** 968–975.
22. Mauger, J. F., A. H. Lichtenstein, L. M. Ausman, S. M. Jalbert, M. Jauhiainen, C. Ehnholm, and B. Lamarche. 2003. Effect of different forms of dietary hydrogenated fats on LDL particle size. *Am. J. Clin. Nutr.* **78:** 370–375.
23. Archer, W. R., B. Lamarche, O. Deriaz, N. Landry, L. Corneau, J. P. Despres, J. Bergeron, P. Couture, and N. Bergeron. 2003. Variations in body composition and plasma lipids in response to a high-carbohydrate diet. *Obes. Res.* **11:** 978–986.
24. Huff, M. W., N. H. Fidge, P. J. Nestel, T. Billington, and B. Watson. 1981. Metabolism of C-apolipoproteins: kinetics of C-II, C-III1 and C-III2, and VLDL-apolipoprotein B in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* **22:** 1235–1246.
25. Nestel, P. J., M. W. Huff, T. Billington, and N. H. Fidge. 1982. Changes in the plasma lipoprotein distribution of apolipoproteins C-II, C-III1, C-III2 and apolipoprotein B after heparin-induced lipolysis. *Biochim. Biophys. Acta.* **712:** 94–102.
26. Huff, M. W., and P. J. Nestel. 1982. Metabolism of apolipoproteins CII, CIII1, CIII2 and VLDL-B in human subjects consuming high carbohydrate diets. *Metabolism.* **31:** 493–498.
27. Kashyap, M. L., L. S. Srivastava, B. A. Hynd, P. S. Gartside, and G. Perisutti. 1981. Quantitation of human apolipoprotein C-III and its subspecie by radioimmunoassay and analytical isoelectric focusing: abnormal plasma triglyceride-rich lipoprotein apolipoprotein C-III subspecie concentrations in hypertriglyceridemia. *J. Lipid Res.* **22:** 800–810.
28. Harduin-Lepers, A., V. Vallejo-Ruiz, M. A. Krzewinski-Recchi, B. Samyn-Petit, S. Julien, and P. Delannoy. 2001. The human sialyltransferase family. *Biochimie.* **83:** 727–737.
29. Paulson, J. C. 1989. Glycoproteins: what are the sugar chains for? *Trends Biochem. Sci.* **14:** 272–276.
30. Millar, J. S. 2001. The sialylation of plasma lipoproteins. *Atherosclerosis.* **154:** 1–13.
31. Cohn, J. S., B. W. Patterson, K. D. Uffelman, J. Davignon, and G. Steiner. 2004. Rate of production of plasma and very-low-density lipoprotein (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male subjects with different body weights and levels of insulin sensitivity. *J. Clin. Endocrinol. Metab.* **89:** 3949–3955.
32. Cohn, J. S., R. Batal, M. Tremblay, H. Jacques, L. Veilleux, C. Rodriguez, O. Mamer, and J. Davignon. 2003. Plasma turnover of HDL apoC-I, apoC-III, and apoE in humans: in vivo evidence for a link between HDL apoC-III and apoA-I metabolism. *J. Lipid Res.* **44:** 1976–1983.
33. Mann, C. J., A. A. Troussard, F. T. Yen, N. Hannouche, J. Najib, J. C. Fruchart, V. Lotteau, P. Andre, and B. E. Bihain. 1997. Inhibitory effects of specific apolipoprotein C-III isoforms on the binding of triglyceride-rich lipoproteins to the lipolysis-stimulated receptor. *J. Biol. Chem.* **272:** 31348–31354.
34. Holdsworth, G., J. Stocks, P. Dodson, and D. J. Galton. 1982. An abnormal triglyceride-rich lipoprotein containing excess sialylated apolipoprotein C-III. *J. Clin. Invest.* **69:** 932–939.
35. Catapano, A. L. 1987. Activation of lipoprotein lipase by apolipoprotein C-II is modulated by the COOH terminal region of apolipoprotein C-III. *Chem. Phys. Lipids.* **45:** 39–47.
36. Tornoci, L., C. A. Scheraldi, X. Li, H. Ide, I. J. Goldberg, and N. A. Le. 1993. Abnormal activation of lipoprotein lipase by non-equilibrating apoC-II: further evidence for the presence of non-equilibrating pools of apolipoproteins C-II and C-III in plasma lipoproteins. *J. Lipid Res.* **34:** 1793–1803.
37. Bukberg, P. R., N. A. Le, H. N. Ginsberg, J. C. Gibson, A. Rubinstein, and W. V. Brown. 1985. Evidence for non-equilibrating pools of apolipoprotein C-III in plasma lipoproteins. *J. Lipid Res.* **26:** 1047–1057.
38. Batal, R., M. Tremblay, P. H. Barrett, H. Jacques, A. Fredenrich, O. Mamer, J. Davignon, and J. S. Cohn. 2000. Plasma kinetics of apoC-III and apoE in normolipidemic and hypertriglyceridemic subjects. *J. Lipid Res.* **41:** 706–718.