Plasma kinetics of apoC-III and apoE in normolipidemic and hypertriglyceridemic subjects

Rami Batal,* Michel Tremblay,* P. Hugh R. Barrett,§ Hélène Jacques,* Alexandre Fredenrich,* Orval Mamer,† Jean Davignon,* and Jeffrey S. Cohn[1,*]

Hyperlipidemia and Atherosclerosis Research Group,* and the McGill University Biomedical Mass Spectrometry Unit,† Montréal, Québec, Canada H2W 1R7, and Department of Medicine,§ University of Western Australia, Perth, Australia 6847

Abstract **Apolipoprotein (apo) C-III and apoE play a central role in controlling the plasma metabolism of triglyceride-rich lipoproteins (TRL). We have investigated the plasma kinetics of total, very low density lipoprotein (VLDL) and high density lipoprotein (HDL) apoC-III and apoE in normolipidemic (NL) (n = 5), hypertriglyceridemic (HTG, n = 5), and Type III hyperlipoproteinemic (n = 2) individuals. Apolipoprotein kinetics were investigated using a primed constant (12 h) infusion of deuterium-labeled leucine. HTG and Type III patients had reduced rates of VLDL apoB-100 catabolism and no evidence of VLDL apoB-100 overproduction. Elevated (3- to 12-fold) total plasma and VLDL apoC-III levels in HTG and Type III patients, although associated with reduced apoC-III catabolism (i.e., increased residence times (RTs)), were mainly due to increased apoC-III production (plasma apoC-III transport rates (TRs, mean ± SEM): (NL) 2.05 ± 0.22 (HTG) 4.90 ± 0.81 ($P < 0.01$), and (Type III) 8.78 mg·kg^{-1}·d^{-1}; VLDL apoC-III TRs: (NL) 1.35 ± 0.23 (HTG) 5.35 ± 0.85 ($P < 0.01$), and (Type III) 7.40 mg·kg^{-1}·d^{-1}). Elevated total plasma and VLDL apoE levels in HTG (2- and 6-fold, respectively) and in Type III (9- and 43-fold) patients were associated with increased VLDL apoE RTs (0.21 ± 0.02, 0.46 ± 0.05 ($P < 0.01$), and 1.21 days, NL vs. HTG vs. Type III, respectively), as well as significantly increased apoE TRs (plasma: (NL) 2.94 ± 0.78 (HTG) 5.80 ± 0.59 ($P < 0.01$) and (Type III) 11.80 mg·kg^{-1}·d^{-1}; VLDL: (NL) 1.59 ± 0.18 (HTG) 4.52 ± 0.61 ($P < 0.01$) and (Type III) 11.95 mg·kg^{-1}·d^{-1}). These results demonstrate that hypertriglyceridemic patients, having reduced VLDL apoB-100 catabolism (including patients with type III hyperlipoproteinemia) are characterized by overproduction of plasma and VLDL apoC-III and apoE.**—Batal, R., M. Tremblay, P. H. R. Barrett, H. Jacques, A. Fredenrich, O. Mamer, J. Davignon, and J. S. Cohn. **Plasma kinetics of apoC-III and apoE in normolipidemic and hypertriglyceridemic subjects.** ***J. Lipid Res.*** **2000.** 41: **706–718.**

Supplementary key words triglyceride • cholesterol • atherosclerosis • type III hyperlipoproteinemia • remnants • stable isotope • metabolism • apoB

ApoC-III and apoE are proteins that play a central role in controlling the plasma metabolism of triglyceride-rich lipoproteins (TRL). ApoC-III is a 8.8 kD glycoprotein, synthesized by the liver and intestine (1). It is associated in circulating blood with all major classes of lipoproteins and has the ability to exchange between TRL and high density lipoproteins (HDL). In normolipidemic (NL) subjects, the majority of apoC-III is bound to HDL, while in hypertriglyceridemic (HTG) subjects, the majority is bound to TRL (2, 3). The importance of apoC-III in regulating plasma TRL metabolism is demonstrated by the fact that: *1*) in the general population, total plasma triglyceride levels are strongly correlated with the concentration of total plasma and TRL apoC-III (4); *2*) individuals with certain apoC-III gene polymorphisms have increased susceptibility to hypertriglyceridemia (5–7); *3*) patients with an inherited deficiency of apoC-III have low plasma triglyceride levels (8, 9); *4*) overexpression of the human apoC-III gene in transgenic mice results in hypertriglyceridemia (10), whereas apoC-III gene knockout mice are hypotriglyceridemic (11); and *5*) in vitro evidence demonstrates that apoC-III has the capacity to inhibit: *a*) the activity of lipoprotein lipase (LPL) (12), *b*) the capacity of TRL to bind to LPL (13), and *c*) the uptake of TRL by the liver (14) through reduced binding of TRL and their remnants to the LDL receptor (LDL-R) (15), the lipolysis-stimulated receptor (LSR) (16), and cell-surface glycosaminoglycans (17), (though not the LDL receptor-related protein (LRP) (18)). Together, these results demonstrate that increased plasma and TRL levels of apoC-III contribute to the reduced lipolysis and receptor-mediated clearance of TRL in HTG individuals.

ApoE, on the other hand, is a 34.2 kD glycoprotein, synthesized by the liver and to a lesser extent by peripheral tissues (19). It is polymorphic in humans and three differ-

Abbreviations: apo, apolipoprotein; GC–MS, gas chromatography–mass spectrometry; HDL, high density lipoprotein; HTG, hypertriglyceridemic; IEF, isoelectric focusing; NL, normolipidemic; pts., patients; RT, residence time; TR, transport rate; TRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein.

[1] To whom correspondence should be addressed.

ent alleles (ε2, ε3, ε4) at a single gene locus are responsible for three major apoE isoforms (E2, E3, E4), of which apoE3 is the most common. ApoE can readily transfer between plasma lipoproteins and, like apoC-III, its concentration in total plasma and TRL is significantly correlated with the level of plasma triglyceride (20–22). ApoE plays a pivotal role in mediating the hepatic recognition and uptake of TRL by serving as a ligand for lipoprotein binding to the LDL-R (23), LRP, and glycosaminoglycans (23). It is also necessary for the normal conversion of VLDL to LDL. This is best exemplified by patients with dysbetalipoproteinemia or type III hyperlipoproteinemia (Type III), who have functionally impaired apoE, reduced clearance and catabolism of plasma TRL, and a pronounced increase in the plasma concentration of apoE-containing TRL remnants. The majority of Type III patients are homozygous for the apoE2 isoform and are characterized by an increase in plasma levels of triglyceride, cholesterol, and apoE, the presence of xanthomas of the palmar creases, and the development of premature cardiovascular disease (24).

Although previous in vivo studies have investigated the plasma kinetics of apoC-III and apoE in HTG and Type III patients, the etiology of their elevated plasma and TRL apoC-III and apoE levels has not been completely elucidated. Plasma apoC-III kinetics have been studied in NL and HTG patients (25–29), and those of apoE have been determined in NL and Type III patients (30–35). In the case of apoC-III, HTG patients were found in one study (29) to have increased rates of apoC-III production and relatively normal rates of apoC-III catabolism. However, in two other studies, HTG patients were characterized by significantly decreased rates of apoC-III catabolism (25, 27). The kinetics of apoE have not been investigated in HTG patients, and those of apoC-III have not been investigated in Type III patients. Furthermore, the kinetics of these proteins have not been studied simultaneously in the same individuals. We have therefore carried out the present study, using an endogenous-labeling primed constant (stable isotope) infusion technique, to simultaneously investigate the plasma kinetics of apoC-III and apoE. Our objective was to determine whether increased levels of plasma apoC-III and apoE in HTG and Type III patients (both having increased levels of very low density lipoprotein (VLDL) apoB-100, due to reduced rates of VLDL apoB-100 catabolism) were due to an increase in their rates of apoC-III and apoE production or a decrease in their rates of catabolism.

METHODS

Study subjects

A total of 12 subjects (11 males and 1 female) were investigated in the present study. Five of these individuals were normolipidemic. They were apparently healthy male subjects, who were selected because they had a fasting plasma triglyceride concentration <2.2 mmol/l, a total plasma cholesterol concentration <5.2 mmol/l, and were within 10% of desirable body weight. Four of them had an apoE 3/3 phenotype and 1 had an apoE 3/2 phenotype. They had no evidence or history of dyslipidemia, diabetes mellitus, or other metabolic disorder, and were not taking medications known to affect plasma lipid metabolism. Five individuals, including the female, were hypertriglyceridemic (HTG) patients recruited from the lipid clinic of the Clinical Research Institute of Montreal. They had plasma triglyceride concentrations >2.2 mmol/l. Four of them were classified as having type IV hyperlipoproteinemia as their LDL cholesterol levels were <3.4 mmol/l. One HTG patient also had an elevated LDL cholesterol level (>4.0 mmol/l) and was classified as having type IIb hyperlipoproteinemia. Two HTG patients had an apoE 3/3 phenotype, one was apoE 4/3, one was apoE 3/2, and one was apoE 4/2. The latter two patients had a familial form of dyslipidemia, characterized by plasma TRL remnant accumulation, as evidenced by VLDL with slow pre-β agarose gel electrophoretic mobility (36). Insufficient data were available to determine whether the remaining 3 patients had a familial form of hyperlipidemia. Although the metabolic basis of hypertriglyceridemia in the HTG patients was therefore different, they were grouped together because they had two critical features in common: *a*) a plasma TG greater than 2.2 mmol/l, and *b*) accumulation in plasma of VLDL, due to reduced VLDL catabolism (see Results). Two male patients were also investigated, who had type III hyperlipoproteinemia (Type III). They were selected on the basis that they were hyperlipidemic, had an apoE 2/2 phenotype, and had β-migrating VLDL (24), as determined by agarose gel electrophoresis. Patients taking lipid-lowering medications (statins or fibrates) were asked to stop their medications 30 days prior to their infusion experiments. All 12 subjects gave informed consent to the study protocol, which was approved by the ethics committee of the Clinical Research Institute of Montreal.

Stable isotope infusion protocol

After a 12-h overnight fast, study subjects were given a primed constant intravenous infusion of deuterium-labeled leucine ([D_3]l-leucine 98%, Cambridge Isotope Laboratories, MA), as previously described (37,38). They were injected via a needle attached to a left forearm vein with 10 μmol per kg body weight of [D_3]l-leucine, dissolved in physiological saline, followed by a 12-h constant infusion (given by peristaltic pump) of 10 μmol [D_3]l-leucine per kg per h. Subjects remained fasted during the infusion but had free access to drinking water. Blood samples (20 ml) were collected from an antecubital vein of the right arm at regular intervals (0, 15, 30, and 45 min, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h) in tubes containing EDTA to a final concentration of 0.1%. Plasma was immediately separated by centrifugation at 3,500 rpm for 15 min at 4°C. An antimicrobial agent (sodium azide) and a protease inhibitor (aprotinin) were added to plasma samples to give a final concentration of 0.02% and 1.67 μg/ml, respectively.

Isolation of lipoproteins and apolipoproteins

VLDL, IDL together with LDL, and HDL were isolated from 5 ml plasma by sequential ultracentrifugation in an XL-90 ultracentrifuge using a 50.4 Ti rotor (Beckman) at 50,000 rpm for 10 h, at densities (d) of 1.006, 1.063, and 1.21 g/ml, respectively. Total lipoproteins were isolated from plasma by ultracentrifugation (50,000 rpm, 10 h) of 1 ml of plasma, adjusted to d 1.25 g/ml with KBr. Lipoproteins were recovered in the supernate by tube-slicing. VLDL apoB-100 was isolated by preparative SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 4–22.5% gradient gels (37). ApoC-III and apoE were isolated from VLDL, HDL, and total plasma lipoproteins (d < 1.25 g/ml fractions) by preparative isoelectric focusing (IEF) on 7.5% polyacrylamide–urea (8 m) gels (pH gradient 4–7) (38). For three subjects, apoE was also prepared by IEF from apoE-containing lipoproteins isolated

from total plasma (1 ml) by immunoaffinity chromatography, using immunopurified polyclonal anti-human apoE antibody. Before IEF separation of HDL and d < 1.25 g/ml apolipoproteins, these fractions were dialyzed against 10 mm ammonium bicarbonate, preincubated with cysteamine (β-mercaptoethylamine, Sigma-Aldrich) in a ratio of 6 mg for every mg of protein for 4 h at 37°C, and then delipidated. The aim of cysteamine treatment was to separate apoE from isoforms of apoA-I, which normally comigrate to the same position on IEF gels. Cysteamine treatment caused an amino group to bind to the single cysteine residue of apoE3, and resulted in two amino groups to be introduced into apoE2 due to the presence of two cysteine residues. ApoA-I and apoE4 were not affected as they did not contain cysteine. Cysteamine-modified apoE2 and apoE3 consequently migrated to a higher position in IEF gels, due to their increased positive charge (38). VLDL samples were delipidated, but were not treated with cysteamine, prior to electrophoresis. Coomassie blue staining was used to identify the position of apolipoproteins in gels after electrophoresis.

Plasma lipids and apolipoproteins

Plasma and lipoprotein fractions were assayed for total (free and esterified) cholesterol and triglyceride with a COBAS MIRA-S automated analyzer (Hoffman-LaRoche) using enzymatic reagents. Plasma and VLDL apoB concentrations were measured with a non-competitive ELISA using immunopurified goat anti-human apoB antibody and horseradish peroxidase-conjugated monoclonal anti-apoB antibody (39). Plasma apoA-I concentrations were measured by nephelometry on a Behring Nephelometer 100 (Behring) using Behring protocol and reagents. Plasma and lipoprotein apoC-III and apoE concentrations were measured with ELISAs developed in our laboratory (3, 22). ApoE phenotypes were determined by isoelectric focusing of delipidated VLDL (38). Total recovery (mean ± SD) of apoC-III and apoE in lipoprotein fractions separated by ultracentrifugation was 89 ± 9% and 63 ± 12%, respectively. ApoC-III and apoE in the bottom fractions, which represented 0.5 ± 0.4% and 13.5 ± 3.1%, respectively (of total plasma apolipoproteins), were considered to be predominantly HDL apolipoproteins and these amounts were mathematically added to HDL for calculation of HDL apoC-III and apoE pool sizes. Lipoprotein apoC-III and apoE concentrations were then corrected for non-recovered apolipoprotein by increasing VLDL, IDL/LDL, and HDL levels by a factor (on average) of 1.1 (for apoC-III) and 1.25 (for apoE).

Determination of isotopic enrichment

Apolipoprotein bands, as well as blank (non-protein containing) gel slices were excised from polyacrylamide gels (VLDL apoB-100 from SDS-PAGE; apoC-III and apoE from IEF gels), as described previously (37, 38). The band corresponding to the major isoform of apoC-III, monosialylated apoC-III (apoC-III_1), was excised and analyzed in all cases. For apoE, the non-sialylated form of apoE3 was analyzed. In subjects with two apoE isoforms, both VLDL apoE bands were analyzed (only one apoE band was present in HDL and d < 1.25 g/ml fractions of these subjects due to the effect of cysteamine treatment). Each slice was added to a borosilicate sample vial containing 600 μl of 6 N HCL and an internal standard of 250 ng norleucine (Sigma-Aldrich) dissolved in 50 μl double-distilled water. Gel slices were hydrolyzed at 110°C for 24 h, cooled to −20°C for 20 min, and centrifuged at 3,500 rpm for 5 min. Free amino acids in the hydrolysate were separated from precipitated polyacrylamide, purified by cation exchange chromatography using AG 50 W-X8 resin (BioRad), and derivatized by treatment with 200 μl of acetyl chloride-acidified 1-propanol (1:5 v/v) for 1 h at 100°C, and 50 μl of heptafluorobutyric anhydride (Supelco) for 20 min at 60°C (37). Plasma amino acids were also separated by cation exchange chromatography and derivatized to allow for the determination of plasma leucine isotopic enrichment. Enrichment of samples with deuterium-labeled leucine was measured by gas chromatography/mass spectrometry (Hewlett-Packard, 5988 GC-MS) using negative chemical ionization and methane as the moderator gas. Selective ion monitoring at m/z = 352 and 349 (ionic species corresponding to derivatized deuterium-labeled and derivatized non-deuterium-labeled leucine, respectively) was performed, and tracer to tracee ratios were derived from isotopic ratios for each sample according to the formula derived by Cobelli, Toffolo, and Foster (40). Tracer to tracee ratios were corrected for background leucine in gel slices (i.e., trace amounts of leucine introduced during the amino acid purification and derivitization procedures) by estimating the amount of leucine in processed blank gel slices relative to the norleucine internal standard. Background leucine represented (mean ± SD) 2.7 ± 0.8% of total leucine recovered for apoB-100 samples and 9.2 ± 3.9% of total leucine recovered for apoC-III and apoE samples.

Kinetic analysis

The primary objective of the present study was to obtain a measure of total plasma apoC-III and apoE production and catabolism. Stable isotope enrichment curves for apoC-III and apoE in the d < 1.25 g/ml fraction were therefore fitted to a three-compartment model using SAAM II computer software (SAAM II institute, WA) (41). The first compartment represented the plasma amino acid precursor pool. The second compartment was a delay compartment, which accounted for the synthesis, assembly and secretion of apolipoproteins. The third compartment was the plasma protein compartment. In those subjects where the VLDL apoB tracer data attained a plateau, we used this value as a measure of the precursor pool enrichment. In those individuals with larger VLDL apoB pool sizes (i.e., HTG and Type III patients, where the curvature of VLDL apoB enrichment curves was not sufficient to define a plateau), average plasma amino acid enrichment was used as the peak enrichment attainable by apoB and the other apolipoproteins. For NL subjects, plasma amino acid enrichments at plateau were within 15% of VLDL apoB enrichments at plateau. This approach may therefore have underestimated the fractional rate of appearance of newly synthesized apolipoproteins by up to 15% in HTG and Type III patients, but this would not have affected the overall conclusions of the study. Modeling of tracer to tracee ratio data resulted in the determination of fractional transport rates (FTR) (i.e., the fraction of protein pools being renewed per day). Residence time (RT) was calculated as the reciprocal of FTR (1/FTR), and transport rate (TR) was calculated (in $mg \cdot kg^{-1} \cdot day^{-1}$) as:

$$TR = \frac{FTR\ (pools/day) \times apolipoprotein\ pool\ size\ (mg)}{body\ weight\ (kg)}$$

where: pool size = plasma concentration (mg/dl) × plasma volume (0.045 liter/kg).

The second objective of this study was to determine kinetic parameters for VLDL apoB-100, as well as apoC-III and apoE in VLDL and HDL. Compartmental analysis with a three-pool model was carried out on the VLDL apoB-100, VLDL apoC-III, VLDL apoE, HDL apoC-III, and HDL apoE data sets separately. This approach provided a measure of the fractional rate of transport of apolipoprotein into and out of VLDL or HDL. It provided an estimate of total apoC-III or apoE entering the VLDL or HDL pools, irrespective of whether the protein was newly synthesized and was derived directly from tissue or whether it was derived from a circulating lipoprotein. Similarly, the calculated residence time was indicative of the time that the apoC-III or apoE was present within its VLDL or HDL pool, irrespective of

whether it was transformed into the apolipoprotein of another lipoprotein exchanged to another lipoprotein, or catabolized by a specific tissue. Transport rates were also expressed in molar units ($nmol \cdot kg^{-1} \cdot day^{-1}$) using a molecular mass of 8,746 daltons for apoC-III, 34,200 daltons for apoE, and 549,000 daltons for apoB-100.

Statistical analysis

The statistical significance of differences between mean values was assessed by paired and unpaired *t*-tests using SigmaStat software (Jandel Scientific, CA). Pearson correlation coefficients (*r*) were calculated to describe the correlation between different kinetic and mass parameters.

RESULTS

Characteristics of study subjects

Plasma lipid and lipoprotein characteristics of the study subjects are shown in **Table 1**. Total plasma triglyceride concentrations were on average 5-fold higher in HTG patients compared to NL subjects. VLDL-TG, VLDL-C, IDL/LDL-TG, but not IDL/LDL-C, concentrations were also significantly increased, and plasma HDL-C, but not apoA-I, concentrations were significantly reduced in HTG patients. Type III patients were severely hypertriglyceridemic and hypercholesterolemic and had higher levels of VLDL-TG, VLDL-C, IDL/LDL-TG (though not IDL/LDL-C) compared to HTG and NL subjects.

Kinetics of plasma VLDL apoB-100

HTG and Type III patients had significantly elevated levels of VLDL apoB-100 (4-fold and 10-fold, respectively) (**Table 2**). These patients had impaired VLDL catabolism rather than VLDL overproduction, which was determined by measuring the plasma kinetics of their VLDL apoB-100. Deuterium-labeled leucine enrichment of VLDL apoB-100 from NL, HTG, and Type III subjects is shown in **Fig. 1**, and kinetic parameters derived by multicompartmental analysis of enrichment curves are shown in Table 2. Delayed catabolism of VLDL apoB-100 was reflected by higher VLDL apoB-100 residence times (RTs) in HTG and Type III patients (5- and 13-fold, respectively) compared to NL subjects. VLDL apoB-100 transport rates (TRs), on the other hand, were not significantly different among the three groups.

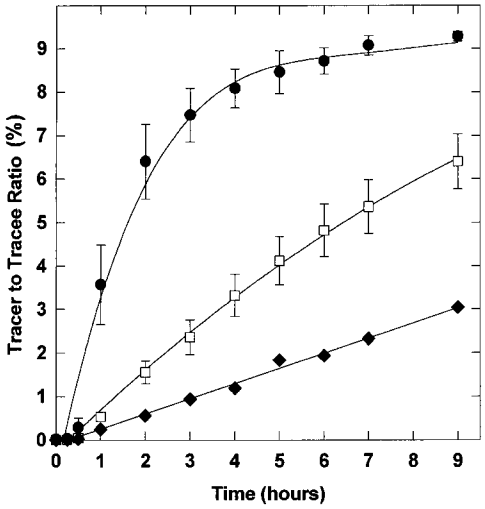

Fig. 1. Enrichment of VLDL apoB-100 with deuterium-labeled leucine in normolipidemic (n = 5) (●–●), hypertriglyceridemic (n = 5) (□–□), and type III (n = 2) (◆–◆) subjects. Error bars represent SEM. Tracer to tracee ratios were normalized to the same level of plateau enrichment to allow for groups to be visually compared.

Kinetics of plasma, VLDL and HDL apoC-III

Plasma and VLDL levels of apoC-III were significantly elevated in HTG and Type III patients compared to NL subjects (**Table 3**). Plasma apoC-III levels were elevated 3- and 6-fold, while VLDL apoC-III levels were elevated 6- and 12-fold in HTG and Type III patients, respectively. HDL apoC-III concentrations were not significantly differ-

TABLE 1. Characteristics of subjects

Variable	Normolipidemic (n = 5)	Hypertriglyceridemic (n = 5)	Type III (n = 2)
Age (yr)	31 ± 2	50 ± 6[a]	44 (31, 56)
BMI (kg/m^2)	24.9 ± 0.5	25.8 ± 1.0	28.3 (26.9, 29.6)
TG (mmol/l)	0.89 ± 0.17	4.36 ± 0.77[b]	12.02 (11.48, 12.56)
TC (mmol/l)	4.00 ± 0.33	5.22 ± 0.58	14.79 (15.59, 13.99)
VLDL-TG (mmol/l)	0.74 ± 0.16	3.81 ± 0.78[b]	11.22 (10.64, 11.80)
VLDL-C (mmol/l)	0.19 ± 0.07	1.72 ± 0.56[a]	12.35 (13.21, 11.48)
IDL/LDL-TG (mmol/l)	0.12 ± 0.02	0.42 ± 0.07[b]	0.58 (0.61, 0.54)
IDL/LDL-C (mmol/l)	2.55 ± 0.27	2.80 ± 0.38	1.96 (1.95, 1.96)
HDL-C (mmol/l)	1.27 ± 0.15	0.70 ± 0.09[a]	0.49 (0.43, 0.55)
ApoB (mg/dl)	79.3 ± 5.9	129.4 ± 7.4[c]	139.3 (145.2, 133.4)
ApoA-I (mg/dl)	125.0 ± 10.1	118.5 ± 5.4	126.4 (124.8, 128.0)

Values are means ± SEM for (n) subjects in each group. For the Type III patients, data for individual patients are given in parentheses together with a mean value. Mean concentrations were calculated from a single value for each subject, which was the average of five measurements made at 3-h intervals during the stable-isotope infusion experiment. BMI, body mass index; TG, triglyceride; TC, total cholesterol; VLDL, very low density lipoprotein; C, cholesterol; IDL/LDL, intermediate density and low density lipoproteins in the $1.006 < d < 1.063$ g/ml fraction; HDL, high density lipoprotein.

[a] $P < 0.05$, [b] $P < 0.01$, and [c] $P < 0.001$, significantly different from normolipidemic subjects by unpaired *t*-test.

TABLE 2. Kinetic parameters for VLDL apoB-100

Subjects	VLDL ApoB-100		
	Concentration	RT	TR
	mg/dl	*days*	*mg·kg^{-1}·d^{-1}*
Normolipidemic (n = 5)	5.9 ± 1.7	0.07 ± 0.02	39.5 ± 5.8
Hypertriglyceridemic (n = 5)	24.9 ± 4.4[a]	0.33 ± 0.06[a]	36.3 ± 6.5
Type III (n = 2)	61.6 (64.0, 59.2)	0.91 (0.77, 1.05)	31.4 (37.4, 25.4)

Values are means ± SEM for (n) subjects in each group. For the type III patients, data for individual patients are given in parentheses with a mean value shown above. Mean VLDL apoB-100 concentrations were calculated from a single value for each subject, which was the average of five measurements made at 3-h intervals during the stable-isotope infusion experiment. RT, residence time; TR, transport rate.

[a] $P < 0.01$; significantly different from normolipidemic subjects by unpaired *t*-test.

ent. Mean data for deuterium-labeled leucine enrichment of apoC-III in VLDL, HDL, and total plasma are shown for NL, HTG and Type III patients in **Fig. 2**. Fractional rates of appearance of newly synthesized apoC-III, reflected by the slopes of enrichment lines, were (without exception) higher in VLDL than in HDL. VLDL and HDL apoC-III enrichment lines were thus significantly different in all cases, providing in vivo evidence for the presence of a significant proportion of plasma apoC-III, which did not exchange and equilibrate between VLDL and HDL. Enrichment of apoC-III in total plasma was intermediate between that of VLDL and HDL (Fig. 2). In NL subjects, where a significant proportion (~60%) of apoC-III was associated with HDL, plasma apoC-III enrichment was more similar to that of HDL than that of VLDL. Conversely, in HTG and Type III patients, where the majority of apoC-III was associated with VLDL, plasma apoC-III enrichment resembled that of VLDL rather than HDL. Kinetic parameters for apoC-III, derived by compartmental analysis, are shown in Table 3. In NL subjects, total plasma apoC-III transport rate was 2.05 ± 0.22 mg·kg^{-1}·day^{-1}, and plasma apoC-III RT was 2.16 ± 0.26 days. These values are comparable to the ones obtained for NL subjects, using exogenous ^{125}I-labeled apoC-III, by Huff et al. (25) (2.6 mg·kg^{-1}·day^{-1} and 1.54 days) and Malmendier et al. (28) (2.3 mg·kg^{-1}·day^{-1} and 1.24 days). RT of apoC-III was 3-fold higher ($P < 0.05$) in HDL than in VLDL in NL subjects, and 1.9-fold ($P < 0.05$) higher in HDL than in VLDL in HTG patients. ApoC-III RTs in total plasma and VLDL were somewhat higher in HTG (1.2- and 1.7-fold) and Type III patients (1.3- and 2.6-fold, respectively) compared to NL subjects, although these differences did not reach statistical significance ($P = 0.12$ and 0.23, NL vs. HTG, respectively). In contrast, TRs for apoC-III in total plasma and VLDL were significantly higher in both HTG (2- and 4-fold) and Type III patients (4- and 5.5-fold, respectively) compared to NL subjects. HDL apoC-III RTs and TRs were not significantly different (Table 3). Elevated levels of plasma and VLDL apoC-III in HTG and Type III patients were thus found to be predominantly due to overproduction of VLDL apoC-III.

Kinetics of plasma, VLDL and HDL apoE

Plasma and VLDL levels of apoE were also significantly elevated in HTG and Type III patients compared to NL subjects (**Table 4**). Plasma apoE levels were elevated 2- and 9-fold, while VLDL apoE levels were elevated 6- and 43-fold in HTG and Type III patients, respectively. HDL apoE concentrations were not significantly different in HTG compared to NL subjects, but were more than 2-fold higher in Type III patients. Mean data for deuterium-labeled leucine enrichment of apoE in VLDL, HDL, and total plasma are shown for NL, HTG, and Type III patients in **Fig. 3**. VLDL apoE enrichment curves were curvilinear in NL and HTG, subjects, but were relatively linear in Type III patients, reflecting a markedly reduced rate of VLDL apoE catabolism in these latter patients. As was the case for apoC-III, fractional rates of appearance of newly synthesized apoE were higher in VLDL than in HDL in all subjects, and the dissimilarity in

TABLE 3. Kinetic parameters for apoC-III

Variable	Normolipidemic (n = 5)	Hypertriglyceridemic (n = 5)	Type III (n = 2)
VLDL ApoC-III			
Concentration (mg/dl)	3.59 ± 0.80	22.36 ± 4.38[a]	44.35 (37.64, 51.05)
RT (days)	1.17 ± 0.11	1.97 ± 0.45	2.99 (1.83, 4.15)
TR (mg·kg^{-1}·d^{-1})	1.35 ± 0.23	5.35 ± 0.85[a]	7.40 (9.26, 5.54)
HDL ApoC-III			
Concentration (mg/dl)	5.34 ± 1.16	3.69 ± 0.52	6.10 (6.96, 5.24)
RT (days)	3.51 ± 0.62	3.80 ± 0.50	4.38 (2.95, 5.80)
TR (mg·kg^{-1}·d^{-1})	0.80 ± 0.21	0.47 ± 0.09	0.73 (1.06, 0.41)
Plasma ApoC-III			
Concentration (mg/dl)	9.47 ± 0.84	28.71 ± 5.12[a]	52.99 (47.37, 58.61)
RT (days)	2.16 ± 0.26	2.66 ± 0.31	2.90 (2.01, 3.79)
TR (mg·kg^{-1}·d^{-1})	2.05 ± 0.22	4.90 ± 0.81[a]	8.78 (10.61, 6.96)

Values are means ± SEM for (n) subjects in each group. For the Type III patients, data for individual patients are given in parentheses together with a mean value. Mean concentrations were calculated from a single value for each subject, which was the average of five measurements made at 3-h intervals during the stable-isotope infusion experiment. RT, residence time; TR, transport rate.

[a] $P < 0.01$, significantly different from normolipidemic subjects by unpaired *t*-test.

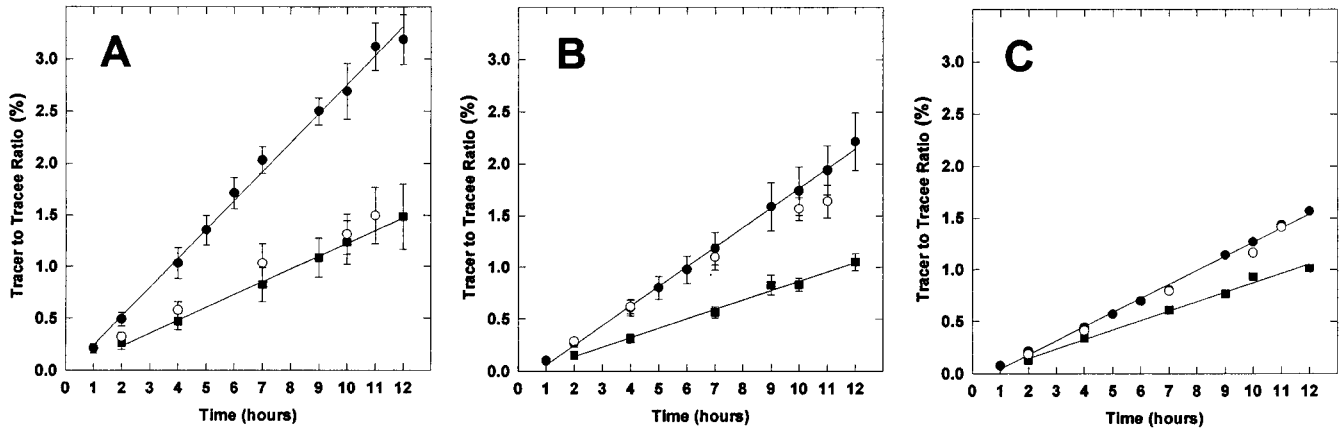

Fig. 2. Enrichment of VLDL (●–●), HDL (■–■) and total plasma apoC-III (○–○) with deuterium-labeled leucine in A: normolipidemic (n = 5) B: hypertriglyceridemic (n = 5), and C: type III (n = 2) subjects. Error bars represent SEM. Tracer to tracee ratios were normalized to the same level of plateau enrichment to allow for groups to be compared visually.

the slopes of these curves provided evidence for the existence of non-exchangeable (or very slowly exchangeable) pools of apoE in human plasma. Rate of enrichment of apoE in total plasma was (like apoC-III) intermediate between that of VLDL and HDL, resembling that of HDL in NL subjects and that of VLDL in Type III patients (Fig. 3). Moreover, enrichment curves for apoE isolated by immunoaffinity chromatography from the plasma of 3 individuals were not different from those of total plasma apoE isolated by ultracentrifugation at d < 1.25 g/ml (**Fig. 4**), confirming that apoE isolated by ultracentrifugation was representative of plasma apoE, despite the stripping of apoE during ultracentrifugal isolation (20, 21). Kinetic parameters for apoE, derived by compartmental analysis, are shown in Table 4. In NL subjects, total plasma apoE transport rate was 2.94 ± 0.78 mg·kg^{-1}·day^{-1}, and plasma apoE RT was 0.85 ± 0.19 days. These values are comparable to the ones obtained for normolipidemic men, with ^{125}I-labeled apoE, by Gregg et al. (31) (4.2 mg·kg^{-1}·day^{-1} and 0.63 days). RT of apoE was 4.5-fold higher ($P < 0.001$) in HDL than in VLDL in NL subjects, and 2.5-fold ($P < 0.05$) higher in HDL than in VLDL in HTG patients. VLDL apoE RTs, but not HDL or total plasma apoE RTs, were significantly ($P < 0.01$) higher in HTG patients compared to NL subjects. Total plasma and VLDL apoE TRs were also significantly increased (2- and 3-fold, respectively), whereas HDL apoE TRs were reduced by about 50% ($P < 0.05$), in HTG patients. RTs of VLDL, HDL and plasma apoE were markedly higher (6-, 4-, and 2-fold, respectively) in Type III patients compared to NL subjects. TRs of apoE in total plasma and VLDL, but not HDL, were also substantially increased in Type III patients, demonstrating that increased production and delayed catabolism contributed to the increase in VLDL and plasma apoE levels in HTG and Type III patients.

TABLE 4. Kinetic parameters for apoE

Variable	Normolipidemic (n = 5)	Hypertriglyceridemic (n = 5)	Type III (n = 2)
VDL ApoE			
Concentration (mg/dl)	0.72 ± 0.08	4.63 ± 0.79[c]	30.97 (32.08, 29.85)
RT[a] (days)	0.21 ± 0.02	0.46 ± 0.05[c]	1.21 (0.99, 1.44)
TR (mg·kg^{-1}·d^{-1})	1.59 ± 0.18	4.52 ± 0.61[c]	11.95 (14.55, 9.35)
HDL-ApoE			
Concentration (mg/dl)	2.99 ± 0.26	2.22 ± 0.35	7.07 (7.00, 7.13)
RT (days)	0.91 ± 0.09	1.14 ± 0.18	3.34 (3.21, 3.47)
TR (mg·kg^{-1}·d^{-1})	1.56 ± 0.24	0.87 ± 0.06[b]	0.95 (0.98, 0.92)
Plasma ApoE			
Concentration (mg/dl)	4.28 ± 0.31	8.03 ± 0.83[c]	40.40 (41.21, 39.58)
RT (days)	0.85 ± 0.19	0.65 ± 0.09	1.55 (1.44, 1.67)
TR (mg·kg^{-1}·d^{-1})	2.94 ± 0.78	5.80 ± 0.59[b]	11.80 (12.91, 10.69)

Values are means ± SEM for (n) subjects in each group. For the Type III patients, data for individual patients are given in parentheses together with a mean value. Mean concentrations were calculated from a single value for each subject, which was the average of five measurements made at 3-h intervals during the stable-isotope infusion experiment. RT, residence time; TR, transport rate.

[a] RT of VLDL apoE in subjects heterozygous for apoE was calculated as the mean of RT values obtained for the two isoforms, and that value was used in calculating TR.

[b] $P < 0.05$; [c] $P < 0.01$, significantly different from normolipidemic subjects by unpaired *t*-test.

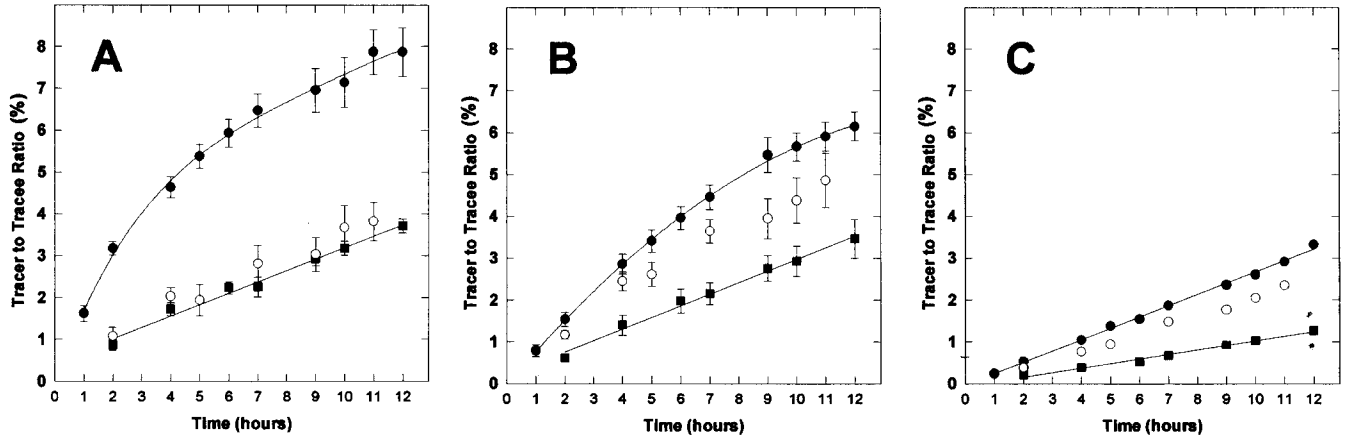

Fig. 3. Enrichment of VLDL (●–●), HDL (■–■) and total plasma apoE (○–○) with deuterium-labeled leucine in A: normolipidemic (n = 5), B: hypertriglyceridemic (n = 5), and C: type III (n = 2) subjects. Error bars represent SEM. Tracer to tracee ratios were normalized to the same level of plateau enrichment to allow for groups to be compared visually.

Relationship between kinetic parameters and plasma concentrations

The relationship between kinetic parameters and plasma concentrations of VLDL and HDL apolipoproteins are presented graphically in **Fig. 5**. Linear regression analyses were carried out with data for the NL and HTG subjects (n = 10), as well as with data for all 12 individuals combined. VLDL apoC-III concentrations were significantly ($P < 0.01$) correlated with VLDL apoC-III TRs ($r = 0.83$ and 0.78, n = 10 and n = 12, respectively) and also VLDL apoC-III RTs ($r = 0.79$ and 0.84). Similarly, VLDL apoE concentrations were significantly ($P < 0.001$) correlated with both VLDL apoE TRs ($r = 0.93$ and 0.94) and RTs ($r = 0.93$ and $r = 0.93$). HDL apoC-III concentrations were significantly ($P < 0.05$) correlated with HDL apoC-III TRs ($r = 0.72$ and 0.72), but not HDL apoC-III RTs ($r = -0.07$ and $r = -0.07$). When Type III patients were not included in the analysis, HDL apoE concentrations were significantly correlated with HDL apoE TRs ($r = 0.68$, $P < 0.05$), but not HDL apoE RTs ($r = 0.24$, $P = 0.49$). When Type III were included in the analysis, HDL apoE concentrations were significantly correlated with HDL apoE RTs ($r = 0.90$, $P < 0.001$), but not HDL apoE TRs ($r = 0.05$, $P = 0.89$).

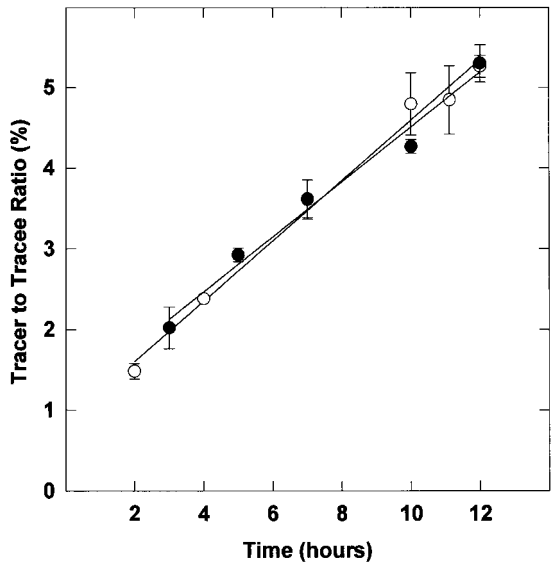

Fig. 4. Deuterium-labeled leucine enrichment of total plasma apoE isolated by ultracentrifugation at d < 1.25 g/ml (○–○) or by immunoaffinity chromatography (●–●) (see Methods). Results (±SEM) are shown for 3 selected subjects. Tracer to tracee ratios were normalized to the same level of plateau enrichment, as in previous figures.

Comparison of VLDL apoB-100, apoC-III, and apoE kinetics

In order to summarize and compare the kinetics of apoB-100, apoC-III, and apoE in VLDL, TRs were expressed in terms of the number of molecules transported per unit time (i.e., in molar units: $nmol \cdot kg^{-1} \cdot day^{-1}$). These data are presented, together with VLDL apolipoprotein RTs, in **Fig. 6**. In NL subjects, VLDL apoB-100, apoE, and apoC-III TRs were 72 ± 11, 47 ± 5, and 155 ± 26 $nmol \cdot kg^{-1} \cdot day^{-1}$, respectively, demonstrating that, on average, for every 2 molecules of VLDL apoB-100 produced by the liver, there was the appearance in VLDL of 1 molecule of newly synthesized apoE and 4 molecules of newly synthesized apoC-III. In contrast, in HTG patients, in whom apoC-III and apoE TRs were significantly increased, 2 molecules of newly synthesized apoE and 10 molecules of newly synthesized apoC-III appeared in VLDL for every molecule of newly synthesized apoB-100. In the case of Type III patients, 6 molecules of newly synthesized apoE and 15 molecules of newly synthesized apoC-III appeared in VLDL for every molecule of newly synthesized apoB-100. In all individuals, VLDL apoC-III RTs were higher than VLDL apoE RTs (NL: $P < 0.001$; HTG: $P < 0.05$), which were in turn higher than VLDL apoB-100 RTs (NL: $P < 0.01$; HTG: $P < 0.01$, Fig. 6B).

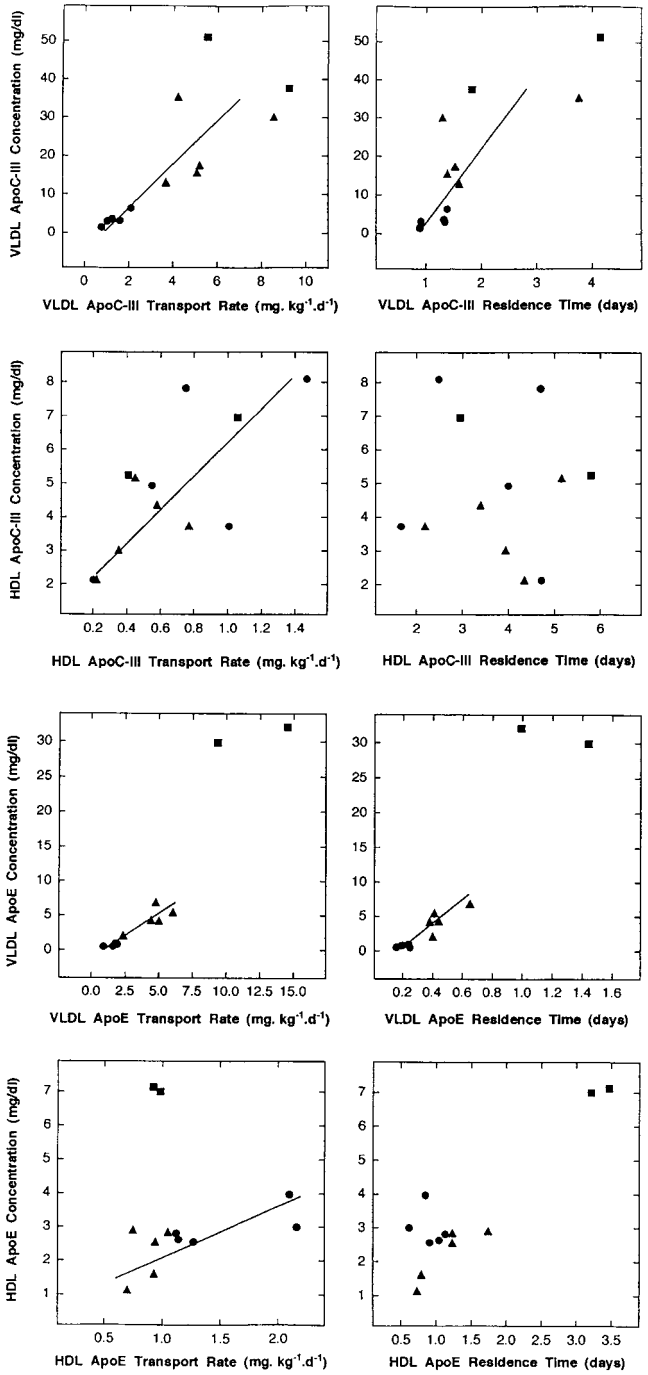

Fig. 5. Relationship between kinetic parameters and plasma concentrations of VLDL and HDL apolipoproteins. Data are indicated for NL subjects (circles), HTG patients (triangles), and for Type III patients (squares). Linear regression lines are shown for those relationships (n = 10, excluding data for Type III patients) which were statistically significant ($P < 0.05$).

DISCUSSION

Elevated levels of plasma and VLDL apoC-III in HTG patients have been found in the present study to be the result of increased apoC-III production rather than decreased apoC-III catabolism. Total plasma apoC-III TRs were 2-fold higher and VLDL apoC-III TRs were 4-fold higher in HTG compared to NL subjects, whereas there was no significant difference in apoC-III RTs (Table 3). Plasma and VLDL apoE TRs were also significantly higher (2- and 3-fold, respectively) in HTG patients. Elevated levels of plasma and VLDL apoE were not, however, due to increased production alone, as HTG patients had significantly decreased rates of VLDL apoE catabolism (Table 4). VLDL apoC-III and apoE overproduction was therefore a characteristic feature of these hypertriglyceridemic patients, which occurred in the absence of increased VLDL apoB-100 production (Table 2). These data support the concept that the rate of apoC-III production can be a primary determinant of plasma triglyceride concentration by causing increased levels of VLDL apoC-III, diminished lipolytic processing and clearance of triglyceride-rich VLDL, and reduced VLDL apoB-100 catabolism. Such a scenario is strongly supported by the fact that: *a*) insulin, fibric acid derivatives, and retinoids alter plasma triglyceride levels by affecting the transcription of the apoC-III gene (42–44), *b*) over- or under-expression of apoC-III in transgenic mice has a marked effect on plasma triglyceride levels (10, 11), and *c*) human apoC-III gene polymorphisms and deficiency states are consistently associated with changes in plasma triglyceride concentration (5–9).

The kinetics of plasma apoC-III and apoE have been investigated in previous studies by monitoring the plasma disappearance of exogenously labeled apolipoproteins. ApoC-III has been investigated after intravenous injection of ^{125}I-labeled VLDL (25–27), ^{125}I-labeled HDL (27), ^{125}I-labeled apoC-III incorporated into HDL (28), or ^{125}I-labeled apoC-III incorporated into total plasma lipoproteins (d $<$ 1.25 g/ml) (29). ApoE has been investigated after intravenous injection of free ^{125}I-labeled apoE (34), or ^{125}I-apoE incorporated into d $<$ 1.21 g/ml plasma lipoproteins (30–33). Irrespective of the form in which radiolabeled apoC-III or apoE was administered, they were found to rapidly exchange and equilibrate with endogenous apolipoproteins in the circulation. In some studies, this exchange was complete, leading to the assumption that radiolabeled apolipoproteins reflected the metabolic characteristics of kinetically homogeneous pools of total plasma apoC-III and apoE. More recent work, however, has suggested that apoC-III and apoE are not fully exchangeable. For example, Bukberg et al. (27) found that when ^{125}I-labeled VLDL was injected intravenously, the specific radioactivity of apoC-III in VLDL remained higher than in HDL, and conversely, when ^{125}I-labeled HDL was injected, apoC-III specific activity remained higher in HDL than in VLDL. In the case of apoE, Gregg et al. (31, 32) found that immediately after injection of ^{125}I-labeled apoE-labeled lipoproteins, the specific activity of apoE in different lipoprotein fractions was equal; however, the rate of disappearance of VLDL ^{125}I-labeled-apoE was more rapid than that of HDL. Exchangeable and non-exchangeable pools of apoC-III and apoE have also been observed in in vitro experiments (45, 46). The present study provides clear evidence of the in vivo existence of kinetically distinct (i.e., non-exchangeable) VLDL and HDL pools of both apoC-III and apoE, as fractional rates of appearance

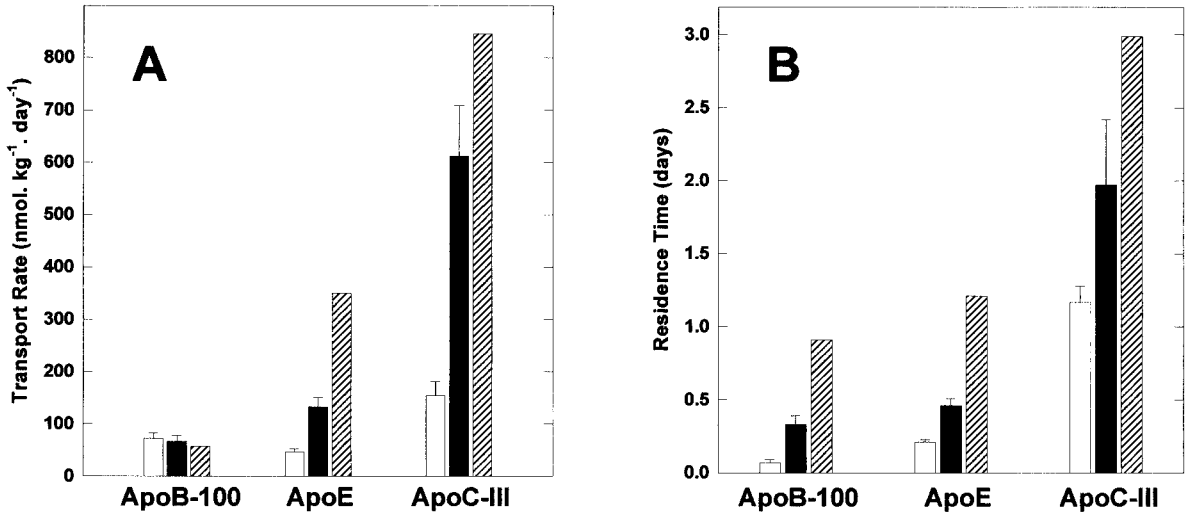

Fig. 6. Comparison of VLDL apolipoprotein (apoB-100, apoC-III, apoE) TRs (A) and RTs (B) in NL (open bars), HTG (filled bars), and Type III subjects (hatched bars). TRs are expressed in molar units, in order to compare them in terms of numbers of molecules newly synthesized. Error bars represent SEMs (n = 5). Mean apoB-100, apoC-III, and apoE kinetic parameters were all significantly different from each other ($P < 0.05$) in NL and HTG patients, except for NL VLDL apoB-100 TR vs. NL VLDL apoE TR ($P = 0.09$).

of newly synthesized apoC-III and apoE were higher in VLDL compared to HDL in all subjects (Figs. 2 and 3). If VLDL and HDL apolipoprotein pools had been fully exchangeable and kinetically homogenous, enrichment curves would have been superimposable. In fact, the rate of exchange of apoC-III and apoE between VLDL and HDL was found to be remarkably low during the infusion experiments, as determined by simple mathematical analysis of simultaneous equations derived from total, VLDL and HDL transport rates (as explained in captions to **Fig. 7** and **Fig. 8**). The maximum possible rate of transport of apoC-III between VLDL and HDL was 0.10 mg· kg^{-1}·day^{-1} in NL subjects and 0.47 mg·kg^{-1}·day^{-1} in HTG subjects (representing 7% and 10% of VLDL apoC-III transport in NL and HTG patients, respectively). For apoE, the maximum possible rate of exchange between VLDL and HDL was calculated to be 0.21 mg·kg^{-1}·day^{-1} in NL subjects (representing 13% of VLDL apoE transport), and was undetectable in HTG subjects. These data indicate that under steady-state fasting conditions, lipoprotein pools of apoC-III and apoE are in large part kinetically distinct. This does not, however, mean that exchange of apoC-III

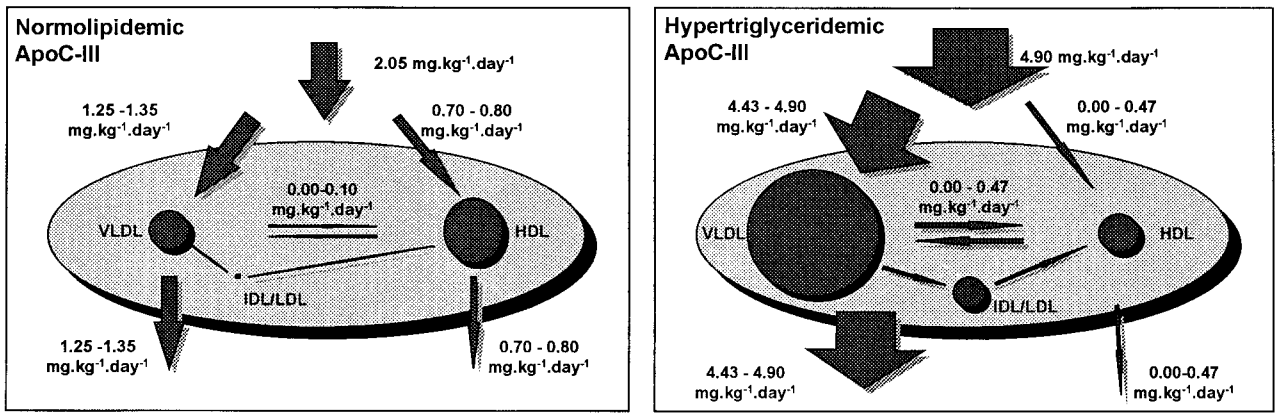

Fig. 7. Schematic representation of plasma apoC-III metabolism in NL and HTG subjects. Elliptical areas represent the plasma. Filled circles represent lipoprotein pools of apoC-III (their size being proportional to the relative mass of apoC-III in each). Transport of apoC-III is depicted by arrows (their size being proportional to the mass of apoC-III transported per kg per day). Minimum and maximum possible rates of transport are shown for each pathway. A maximum value in one pathway usually corresponds to a minimum value for the alternate pathway, in order to be an appropriate solution. These values were determined by solving a set of simultaneous equations, in which: *a)* total plasma apoC-III transport was assumed to equal the sum of direct production of apoC-III into VLDL and HDL (i.e., it was assumed that no newly synthesized apoC-III was secreted directly into the IDL/LDL fraction); *b)* apoC-III transport into VLDL was equal to the sum of direct apoC-III production into VLDL and transfer of apoC-III from HDL; and *c)* apoC-III transport into HDL was equal to the sum of direct apoC-III production into HDL and transfer of apoC-III from VLDL (i.e., it was assumed that apoC-III transport into and out of IDL/LDL was negligible; in actual fact, IDL/LDL apoC-III represented 6% and 9% of total plasma apoC-III mass in NL and HTG patients, respectively).

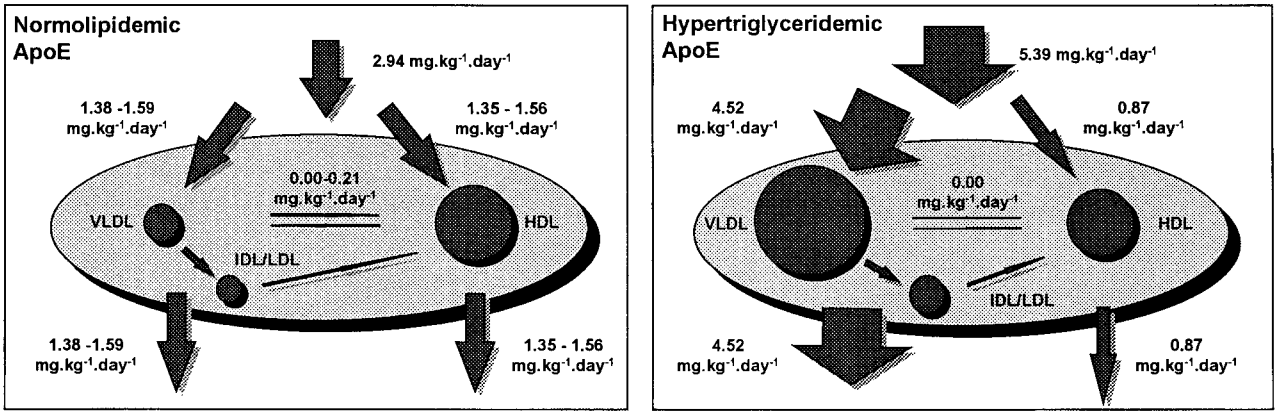

Fig. 8. Schematic representation of plasma apoE metabolism in NL and HTG subjects. Elliptical areas represent the plasma. Filled circles represent lipoprotein pools of apoE (their size being proportional to the relative mass of apoE in each). Transport of apoE is depicted by arrows (their size being proportional to the mass of apoE transported per kg per day). Minimum and maximum possible rates of transport are shown for each pathway, and were determined by solving simultaneous equations, and making certain assumptions, as described for apoC-III in the caption of Fig. 7. (It was assumed that apoE transport into and out of IDL/LDL was negligible; in actual fact, IDL/LDL apoE represented 13% and 15% of total plasma apoE mass in NL and HTG patients, respectively.)

or apoE between plasma lipoproteins is quantitatively unimportant under physiological non-steady state conditions, as evidenced by the fact that during alimentary lipemia, apoE can redistribute from HDL to VLDL (47), and can move from TRL to HDL as a result of postheparin lipolysis (48).

An additional feature of the analyses presented in Figs. 7 and 8 is that in both NL and HTG subjects a significant proportion of total plasma apoC-III and apoE was produced on VLDL. In the case of apoC-III, VLDL production accounted for about 60% and 90% of total plasma apoC-III production in NL and HTG patients, respectively. For apoE, VLDL production accounted for about 50% and 80% of total plasma apoE production, respectively. Hepatic synthesis and secretion of apoC-III and apoE on nascent VLDL and HDL has been demonstrated by studies with perfused rat livers and isolated human hepatocytes (49–52). The relative importance of different lipoproteins in the tissue output of these apolipoproteins has not however been clearly defined. Evidence has been presented showing that apoC-III and apoE are almost completely associated with nascent VLDL in highly purified Golgi fractions of rat hepatocytes (53). Fazio and Yao (54) have shown that stimulation of lipogenesis and increased production of apoB-containing lipoproteins in HepG2 cells causes increased intracellular association of apoE with VLDL and decreased association with HDL, but no increase in total apoE production. Miller et al. (35) have in turn demonstrated that apoE is predominantly produced on VLDL in the fed state. These results together suggest that: *a*) under normal fasting conditions, a significant proportion of newly synthesized apoE and apoC-III is secreted on VLDL, and *b*) this proportion increases in the fed state and in HTG patients with elevated levels of VLDL.

We have found that the TR of VLDL apoC-III, expressed in molar terms, was higher than that of VLDL apoB-100, which was in turn higher than VLDL apoE in all groups (Fig. 6). In NL subjects, each newly synthesized VLDL particle was therefore secreted into plasma associated with several (2 on average) apoC-III molecules, but not always with apoE. In HTG and Type III patients, a newly synthesized VLDL particle was secreted into plasma containing several molecules of both apoC-III (10 and 15 molecules, respectively) and apoE (2 and 6 molecules, respectively). At the same time, RTs of VLDL apoC-III and E were higher than those of VLDL apoB-100, even in the case of HTG and Type III patients (Fig. 6), who had significantly reduced catabolism of VLDL apoB-100. This demonstrates that in the fasted state: *a*) apoE and apoC-III molecules transfer between VLDL particles, and during their "life-time" in the circulation, they reside on more than one VLDL particle, and *b*) as each VLDL particle is cleared from the circulation, its apoC-III and apoE components are not necessarily catabolized with it.

Significantly, we have found that plasma and VLDL apoC-III production was also increased in two patients with Type III HLP. These patients had plasma triglyceride levels which were 3-fold higher, plasma and VLDL apoC-III levels which were 2-fold higher, and plasma and VLDL apoC-III TRs which were 80% and 40% higher, compared to HTG patients. To our knowledge, apoC-III kinetics have not been previously investigated in Type III patients. This is in large part because the primary molecular defect responsible for this disorder resides within apoE. It is well accepted that homozygosity for apoE2 (or the presence of other genetically impaired forms of apoE) can cause an impairment in TRL conversion to LDL and a significant reduction in receptor-mediated recognition and uptake of TRL remnants by the liver (34). Hypertriglyceridemia and hypercholesterolemia in Type III patients are therefore a consequence of accumulation in plasma of apoE-rich TRL remnants. However, not all patients (less than 2%) with an

apoE 2/2 phenotype have Type III, most are in fact hypocholesterolemic, due to decreased levels of LDL (55). Development of overt hyperlipidemia is dependent on the presence of other genetic or environmental factors, such as hypothyroidism, pregnancy, diabetes, estrogen withdrawal, or obesity (34). Based on the present results, we suggest that a biochemical mechanism common to many of these factors might be the overproduction of apoC-III, which leads to increased amounts of apoC-III in TRL, decreased TRL lipolysis, and further reduction in hepatic recognition and uptake of apoE2-containing TRL remnants. Overproduction of VLDL apoC-III thus stresses a system, which is already burdened by the presence of dysfunctional apoE. Whether the simultaneous occurrence of an apoC-III gene polymorphism (causing increased apoC-III production) and an apoE 2/2 phenotype in single individuals causes an increased frequency in the clinical expression of Type III HLP, analogous to that caused by an LDL receptor defect (56) or an apoB defect (57), remains to be determined.

Consistent with previous studies demonstrating that Type III patients have significantly impaired catabolism of VLDL apoE (30) and VLDL apoB-100 (58, 59), we observed that VLDL apoB-100 and VLDL apoE RTs in Type III patients were 3-fold higher compared to HTG patients and 13- and 6-fold higher, respectively, compared to NL subjects (Tables 2 and 4). Interestingly, VLDL apoE TRs were also 2.5-fold higher relative to HTG patients and 7.5-fold higher relative to NL subjects. Thus, although reduced apoE catabolism is generally regarded as the primary mechanism responsible for accumulation of apoE in the plasma of Type III patients, our data suggest that apoE overproduction (predominantly in the form of VLDL apoE) is an important factor contributing to increased plasma and VLDL apoE levels. Overproduction of apoE has similarly been observed in a patient with dominant expression of Type III hyperlipoproteinemia, due to the presence of an apoE mutant, apoE-1 ($Lys^{146}\rightarrow Glu$) (60). Increased apoE production in Type III patients is probably a secondary effect, whereby the liver synthesizes and secretes increased amounts of apoE in response to reduced hepatic uptake of remnant lipoproteins and reduced flux of lipids into the liver.

In conclusion, the present results have shown that: *1*) apoC-III and apoE are not fully exchangeable between VLDL and HDL, and they thus represent kinetically distinct pools of apolipoprotein; *2*) in both normolipidemic and hypertriglyceridemic subjects, a significant proportion (50% or more) of total plasma apoC-III and apoE production is accounted for by apolipoprotein production into VLDL; *3*) increased levels of plasma and VLDL apoC-III in hypertriglyceridemic subjects, having decreased VLDL apoB-100 catabolism, are mainly the result of an increase in apoC-III production rather than a decrease in apoC-III catabolism; *4*) increased levels of plasma and VLDL apoE in hypertriglyceridemic patients are associated with increased VLDL apoE residence times, as well as significantly increased rates of apoE production; *6*) apoE overproduction, and not just reduced apoE catabolism, is an important factor contributing to increased plasma and VLDL apoE levels in Type III patients, and *7*) plasma and VLDL apoC-III production is increased in patients with type III hyperlipoproteinemia, which may be an important biochemical mechanism responsible for overt hyperlipidemia in individuals with an apoE 2/2 phenotype. These results provide evidence that normal levels of plasma apoC-III and apoE production are crucial for maintaining a normal plasma lipid profile, and that therapeutic intervention aimed at reducing apoC-III production is a logical strategy for the treatment of hypertriglyceridemia.

This study was funded by an MRC operating grant (MT-14684) to J. S. Cohn. R. Batal received a scholarship and J. S. C. was supported by a grant-in-aid from the Heart and Stroke Foundation of Québec. The financial support of Parke-Davis and Merck Frosst was also critical for this work. We would particularly like to thank Nancy Doyle for her excellent technical assistance, as well as Dr. Madeleine Roy, Denise Dubreuil, and the nurses of the Lipid Clinic of the Clinical Research Institute of Montréal for their help in the clinic.

Manuscript received 1 April 1999, in revised form 11 November 1999, and in revised form 4 February 2000.

REFERENCES

1. Lenich, C., P. Brecher, S. Makrides, A. Chobanian, and V. I. Zannis. 1988. Apolipoprotein gene expression in the rabbit: abundance, size, and distribution of apolipoprotein mRNA species in different tissues. *J. Lipid Res.* **29:** 755–764.
2. Schonfeld, G., P. K. George, J. Miller, P. Reilly, and J. Witztum. 1979. Apolipoprotein C-II and C-III levels in hyperlipoproteinemia. *Metabolism.* **28:** 1001–1010.
3. Fredenrich, A., L-M. Giroux, M. Tremblay, L. Krimbou, J. Davignon, and J. S. Cohn. 1997. Plasma lipoprotein distribution of apoC-III in normolipidemic and hypertriglyceridemic subjects: comparison of the apoC-III to apoE ratio in different lipoprotein fractions. *J. Lipid Res.* **38:** 1421–1432.
4. Curry, M. D., W. J. McConathy, J. D. Fesmire, and P. Alaupovic. 1980. Quantitative determination of human apolipoprotein C-III by electroimmunoassay. *Biochim. Biophys. Acta.* **617:** 503–513.
5. Dammerman, M., L. A. Sandkuijl, J. L. Halaas, W. Chung, and J. L. Breslow. 1993. An apolipoprotein CIII haplotype protective against hypertriglyceridemia is specified by promoter and 3′ untranslated region polymorphisms. *Proc. Natl. Acad. Sci. USA.* **90:** 4562–4566.
6. Surguchov, A. P., G. P. Page, L. Smith, W. Patsch, and E. Boerwinkle. 1996. Polymorphic markers in apolipoprotein C-III gene flanking regions and hypertriglyceridemia. *Arterioscler. Thromb. Vasc. Biol.* **16:** 941–947.
7. Ribalta, J., A. E. La Ville, J. C. Vallvé, S. Humphries, P. R. Turner, and L. Masana. 1997. A variation in the apolipoprotein C-III gene is associated with an increased number of circulating VLDL and IDL particles in familial combined hyperlipidemia. *J. Lipid Res.* **38:** 1061–1069.
8. Forte, T. M., A. V. Nichols, R. M. Krauss, and R. A. Norum. 1984. Familial apolipoprotein AI and apolipoprotein CIII deficiency. Subclass distribution, composition, and morphology of lipoproteins in a disorder associated with premature atherosclerosis. *J. Clin. Invest.* **74:** 1601–1613.
9. Von Eckardstein A., H. Holz, M. Sandkamp, W. Weng, H. Funke, and G. Assmann. 1991. Apolipoprotein C-III ($Lys_{58}\rightarrow Glu$). Identification of an apolipoprotein C-III variant in a family with hyperalphalipoproteinemia. *J. Clin. Invest.* **87:** 1724–1731.
10. Ito, Y., N. Azrolan, A. O'Connell, A. Walsh, and J. L. Breslow. 1990. Hypertriglyceridemia as a result of human apoCIII gene expression in transgenic mice. *Science.* **249:** 790–793.

11. Maeda, N., H. Li, D. Lee, P. Oliver, S. H. Quarfordt, and J. Osada. 1994. Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. *J. Biol. Chem.* **269:** 23610–23616.
12. Brown, W. V., and M. L. Baginsky. 1972. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochim. Biophys. Acta.* **46:** 375–382.
13. Van Barlingen, H. H. J. J., H. de Jong, D. W. Erkelens, and T. W. A. de Bruin. 1996. Lipoprotein lipase-enhanced binding of human triglyceride-rich lipoproteins to heparan sulfate: modulation by apolipoprotein E and apolipoprotein C. *J. Lipid Res.* **37:** 754–763.
14. Windler, E., and R. J. Havel. 1985. Inhibitory effects of C apolipoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver. *J. Lipid Res.* **26:** 556–563.
15. Clavey, V., S. Lestavel-Delattre, C. Copin, J. M. Bard, and J. C. Fruchart. 1995. Modulation of lipoprotein B binding to the LDL receptor by exogenous lipids and apolipoproteins CI, CII, CIII and E. *Arterioscler. Thromb. Vasc. Biol.* **15:** 963–971.
16. 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.
17. 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.
18. Weisgraber, K. H., R. W. Mahley, R. C. Kowal, J. Herz, J. L. Goldstein, and M. S. Brown. 1990. Apolipoprotein C-I modulates the interaction of apolipoprotein E with β-migrating very low density lipoproteins (β-VLDL) and inhibits binding of β-VLDL to low density lipoprotein receptor-related protein. *J. Biol. Chem.* **265:** 22453–22459.
19. Weisgraber, K. H. 1994. Apolipoprotein E: structure-function relationships. *Adv. Prot. Chem.* **45:** 249–302.
20. Blum, C. B., L. Aron, and R. Sciacca. 1980. Radioimmunoassay studies of human apolipoprotein E. *J. Clin. Invest.* **66:** 1240–1250.
21. Havel, R. J., L. Kotite, J-L. Vigne, J. P. Kane, P. Tun, N. Phillips, and G. C. Chen. 1980. Radioimmunoassay of human arginine-rich apolipoprotein, apoprotein E. *J. Clin. Invest.* **66:** 1351–1362.
22. Cohn, J. S., M. Tremblay, M. Amiot, D. Bouthillier, M. Roy, J. Genest, Jr., and J. Davignon. 1996. Plasma concentration of apolipoprotein E in intermediate-sized remnant-like lipoproteins in normolipidemic and hyperlipidemic subjects. *Arterioscler. Thromb. Vasc. Biol.* **16:** 149–159.
23. Mahley, R. W., and Y. Huang. 1999. Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. *Curr. Opin. Lipidol.* **10:** 207–217.
24. Mahley, R. W., and S.C. Rall, Jr. 1989. Type III hyperlipoproteinemia (dysbeta-lipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill Publishing Co., New York, NY. 1195–1213.
25. Huff, M. W., N. H. Fidge, P. J. Nestel, T. Billington, and B. Watson. 1981. Metabolism of C-apolipoproteins: kinetics of C-II, C-III_1 and C-III_2, and VLDL-apolipoprotein B in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* **22:** 1235–1246.
26. Huff, M. W., and P. J. Nestel. 1982. Metabolism of apolipoproteins CII, $CIII_1$, $CIII_2$ and VLDL-B in human subjects consuming high carbohydrate diets. *Metabolism.* **31:** 493–498.
27. Bukberg, P. R., N-A. Le, H. N. Ginsberg, J. G. 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.
28. Malmendier, C. L., J. F. Lontie, G. A. Grutman, and C. Delcroix. 1988. Metabolism of apolipoprotein C-III in normolipemic human subjects. *Atherosclerosis.* **69:** 51–59.
29. Malmendier, C. L., J. F. Lontie, C. Delcroix, D. Y. Dubois, T. Magot, and L. De Roy. 1989. Apolipoproteins C-II and C-III metabolism in hypertriglyceridemic patients. Effect of a drastic triglyceride reduction by combined diet restriction and fenofibrate administration. *Atherosclerosis.* **77:** 139–149.
30. Gregg, R. E., L. A. Zech, E. J. Schaefer, and H. B. Brewer, Jr. 1981. Type III hyperlipoproteinemia: defective metabolism of an abnormal apolipoprotein E. *Science.* **211:** 584–586.
31. Gregg, R. E., L. A. Zech, E. J. Schaefer, and H. B. Brewer, Jr. 1984. Apolipoprotein E metabolism in normolipidemic human subjects. *J. Lipid Res.* **25:** 1167–1176.
32. Gregg, R. E., L. A. Zech, E. J. Schaefer, D. Stark, D. Wilson, and H. B. Brewer, Jr. 1986. Abnormal in vivo metabolism of apolipoprotein E4 in humans. *J. Clin. Invest.* **78:** 815–821.
33. Schaefer, E. J., R. E. Gregg, G. Ghiselli, T. M. Forte, J. M. Ordovas, L. A. Zech, and H. B. Brewer, Jr. 1986. Familial apolipoprotein E deficiency. *J. Clin. Invest.* **78:** 1–14.
34. Ghiselli, G., Y. Beigel, M. Soma, and A. M. Gotto, Jr. 1986. Plasma catabolism of human apolipoprotein E isoproteins: lack of conversion of the doubly sialilated form to the asialo form in plasma. *Metabolism.* **35:** 399–403.
35. Millar, J. S., A. H. Lichtenstein, G. G. Dolnikowski, J. M. Ordovas, and E. J. Schaefer. 1998. Proposal of a multicompartmental model for use in the study of apolipoprotein E metabolism. *Metabolism.* **47:** 922–928.
36. Davignon, J., J. Dallongeville, G. Roederer, M. Roy, J. C. Fruchart, A. M. Kessling, D. Bouthillier, and S. Lussier-Cacan. 1991. A phenocopy of the type III dyslipoproteinemia occuring in a candidate family for a putative apoE receptor defect. *Ann. Med.* **23:** 161–167.
37. Cohn, J. S., D. A. Wagner, S. D. Cohn, J. S. Millar, and E. J. Schaefer. 1990. Measurement of very low density and low density lipoprotein apolipoprotein (apo) B-100 and high density lipoprotein apo A-I production in human subjects using deuterated leucine. Effect of fasting and feeding. *J. Clin. Invest.* **85:** 804–811.
38. Batal, R., M. Tremblay, L. Krimbou, O. Mamer, J. Davignon, J. Genest, Jr., and J. S. Cohn. 1998. Familial HDL deficiency characterized by hypercatabolism of mature apoA-I but not proapoA-I. *Arterioscler. Thromb. Vasc. Biol.* **18:** 655–664.
39. Albers, J. J., M. S. Lodge, and L. K. Curtiss. 1989. Evaluation of a monoclonal antibody-based enzyme-linked immunosorbent assay as a candidate reference method for the measurement of apolipoprotein B-100. *J. Lipid Res.* **30:** 1445–1458.
40. Cobelli, C., G. Toffolo, and D. Foster. 1992. Tracer-to-tracee ratio for analysis of stable isotope tracer data: link with radioactive kinetic formalism. *Am. J. Physiol.* **262:** E968–E975.
41. Riches, F. M., G. F. Watts, F. M. van Bockxmeer, J. Hua, S. Song, S. E. Humphries, and P. J. Talmud. 1998. Apolipoprotein B signal peptide and apolipoprotein E genotypes as determinants of the hepatic secretion of VLDL apoB in obese men. *J. Lipid Res.* **39:** 1752–1758.
42. Chen, M., J. L. Breslow, W. Li, and T. Leff. 1994. Transcriptional regulation of the apoC-III gene by insulin in diabetic mice: correlation with changes in plasma triglyceride levels. *J. Lipid Res.* **35:** 1918–1924.
43. Staels, B., N. Vu-Dac, V. A. Kosykh, R. Saladin, J. C. Fruchart, J. Dallongeville, and J. Auwerx. 1995. Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase. *J. Clin. Invest.* **95:** 705–712.
44. Vu-Dac, N., P. Gervois, I. P. Torra, J-C. Fruchart, V. Kosykh, T. Kooistra, H. M. G. Princen, J. Dallongeville, and B. Staels. 1998. Retinoids increase human apoC-III expression at the transcriptional level via the retinoid X receptor. Contribution to the hypertriglyceridemic action of retinoids. *J. Clin. Invest.* **102:** 625–632.
45. 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.
46. Rubinstein, A., J. C. Gibson, H. N. Ginsberg, and W. V. Brown. 1986. In vitro metabolism of apolipoprotein E. *Biochim. Biophys. Acta.* **879:** 355–361.
47. Blum, C. B. 1982. Dynamics of apolipoprotein E metabolism in humans. *J. Lipid Res.* **23:** 1308–1316.
48. Rubinstein, A., J. C. Gibson, J. R. Paterniti, Jr., G. Kakis, A. Little, H. N. Ginsberg, and W. V. Brown. 1985. Effect of heparin-induced lipolysis on the distribution of apolipoprotein E among lipoprotein subclasses. Studies with patients deficient in hepatic triglyceride lipase and lipoprotein lipase. *J. Clin. Invest.* **75:** 710–721.
49. Noel, S-P., and D. Rubinstein. 1974. Secretion of apolipoproteins

in very low density and high density lipoproteins by perfused rat liver. *J. Lipid Res.* **15:** 301–308.
50. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667–680.
51. Hamilton, R. L., J. S. Wong, L. S. S. Guo, S. Krisnas, and R. J. Havel. 1990. Apolipoprotein E localization in rat hepatocytes by immunogold labeling of cryothin sections. *J. Lipid Res.* **31:** 1589–1603.
52. Fazio, S., Z. Yao, B. J. McCarthy, and S. C. Rall, Jr. 1992. Synthesis and secretion of apolipoprotein E occur independently of synthesis and secretion of apolipoprotein B-containing lipoproteins in HepG2 cells. *J. Biol. Chem.* **267:** 6941–6945.
53. Hamilton, R. L., A. Moorehouse, and R. J. Havel. 1991. Isolation and properties of nascent lipoproteins from highly purified rat hepatocytic Golgi fractions. *J. Lipid Res.* **32:** 529–543.
54. Fazio, S., and Z. Yao. 1995. The enhanced association of apolipoprotein E with apolipoprotein B-containing lipoproteins in serum-stimulated hepatocytes occurs intracellularly. *Arterioscler. Thromb. Vasc. Biol.* **15:** 593–600.
55. Rall, S. C., Jr., K. H. Weisgraber, T. L. Innerarity, R. W. Mahley, and G. Assmann. 1983. Identical structural and receptor binding defects in apolipoprotein E2 in hypo-, normo-, and hypercholesterolemic dysbetalipoproteinemia. *J. Clin. Invest.* **71:** 1023–1031.
56. Hopkins, P. N., L. L. Wu, M. C. Schumacher, M. Emi, R. M. Hegele, S. C. Hunt, J-M Lalouel, and R. R. Williams. 1991. Type III dyslipoproteinemia in patients heterozygous for familial hypercholesterolemia and apolipoprotein E2. Evidence for a gene-gene interaction. *Arterioscler. Thromb.* **11:** 1137–1146.
57. Groenewegen, W. A., E. S. Krul, M. R. Averna, J. Pulai, and G. Schonfeld. 1994. Dysbetalipoproteinemia in a kindred with hypobetalipoproteinemia due to mutations in the genes for apoB (apoB-70.5) and apoE (apoE2). *Arterioscler. Thromb.* **14:** 1695–1704.
58. Chait, A., W. R. Hazzard, J. J. Albers, R. P. Kushwaha, and J. D. Brunzell. 1978. Impaired very low density lipoprotein and triglyceride removal in broad beta disease: comparison with endogenous hypertriglyceridemia. *Metabolism.* **27:** 1055–1066.
59. Stalenhoef, A. F. H., M. J. Malloy, J. P. Kane, and R. J. Havel. 1986. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in patients with familial dysbetalipoproteinemia. *J. Clin. Invest.* **78:** 722–728.
60. Mann, W. A., P. Lohse, R. E. Gregg, R. Ronan, J. M. Hoeg, L. A. Zech, and H. B. Brewer, Jr. 1995. Dominant expression of type III hyperlipoproteinemia. Pathophysiological insights derived from the structural and kinetic characteristics of apoE-1 (Lys^{164}→Glu). *J. Clin. Invest.* **96:** 1100–1107.