# Plasma kinetics of VLDL and HDL apoC-I in normolipidemic and hypertriglyceridemic subjects

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**Abstract ApoC-I has several different lipid-regulating functions including, inhibition of receptor-mediated uptake of plasma triglyceride-rich lipoproteins, inhibition of cholesteryl ester transfer activity, and mediation of tissue fatty acid uptake. Since little is known about the rate of production and catabolism of plasma apoC-I in humans, the present study was undertaken to determine the plasma kinetics of VLDL and HDL apoC-I using a primed constant (12 h) intravenous infusion of deuterium-labeled leucine. Data were obtained for 14 subjects: normolipidemics (NL, n = 4), hypertriglyceridemics (HTG, n = 4) and combined hyperlipidemics (CHL, n = 6). Plasma VLDL triglyceride (TG) levels were 0.59 ± 0.03, 4.32 ± 0.77 ($P < 0.01$ vs. NL), and 2.20 ± 0.39 mmol/l ($P < 0.01$ vs. NL), and plasma LDL cholesterol (LDL-C) levels were 2.34 ± 0.22, 2.48 ± 0.26, and 5.35 ± 0.48 mmol/l ($P < 0.01$ vs. NL), respectively. HTG and CHL had significantly ($P < 0.05$) increased levels of total plasma apoC-I (12.5 ± 1.2 and 12.4 ± 1.3 mg/dl, respectively) versus NL (7.9 ± 0.6 mg/dl), due to significantly ($P < 0.01$) elevated levels of VLDL apoC-I (5.8 ± 0.8 and 4.5 ± 0.8 vs. 0.3 ± 0.1 mg/dl). HTG and CHL also had increased rates of VLDL apoC-I transport (i.e., production) versus NL: 2.29 ± 0.34 and 3.04 ± 0.53 versus 0.24 ± 0.11 mg/kg.day ($P < 0.01$), with no significant change in VLDL apoC-I residence times (RT): 1.16 ± 0.12 versus 0.69 ± 0.06 versus 0.74 ± 0.17. Although HDL apoC-I concentrations were not significantly lower in HTG and CHL versus NL, HDL apoC-I rates of transport were inversely related to plasma and VLDL-TG levels ($r = -0.63$ and $-0.62$, respectively, $P < 0.05$). Our results demonstrate that increased levels of plasma and VLDL apoC-I in hypertriglyceridemic subjects (with or without elevated LDL-C levels) are associated with increased levels of plasma VLDL apoC-I production.**—Cohn, J. S., M. Tremblay, R. Batal, H. Jacques, L. Veilleux, C. Rodriguez, L. Bernier, O. Mamer, and J. Davignon. **Plasma kinetics of VLDL and HDL apoC-I in normolipidemic and hypertriglyceridemic subjects. *J. Lipid Res.* 2002.** 43: **1680–1687.**

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Apolipoprotein (apo)C-I is a plasma protein that plays an important role in regulating the plasma metabolism of human triglyceride-rich lipoproteins (TRL) (1, 2). It is a 57-amino acid protein (6613 Da mol wt.), containing a high percentage of lysine (16 mol%), but no histidine, tyrosine, cysteine, or carbohydrate (3, 4). It is synthesized with a 26-residue signal peptide, which is cleaved cotranslationally in the rough endoplasmic reticulum (5). The *APOCI* gene (~4.7 kb) is located on chromosome 19 together with the *APOE* and pseudo-*APOCI′* genes, and is situated in the same transcriptional orientation 5.3 kb downstream from the *APOE* gene. The major source of plasma apoC-I is the liver, but it is also synthesized by the lung, skin, testes, and spleen (6).

Although the biological function of apoC-I in humans has not been completely elucidated, in vitro experiments have demonstrated that apoC-I has the capacity to: *a*) activate LCAT (7–9); *b*) inhibit lipoprotein lipase (10, 11), hepatic lipase (12, 13), and phospholipase $A_2$ (14); and *c*) inhibit cholesterol ester transfer activity (15, 16). ApoC-I has also been shown to play an important role in plasma TRL metabolism by inhibiting the binding and/or uptake of triglyceride emulsions or VLDL by the LDL receptor (17, 18), the LDL receptor-related protein (LRP) (19, 20), and the VLDL receptor (21). Inhibition of lipoprotein binding to lipoprotein receptors is believed to be due to the ability of apoC-I to displace significant amounts of apoE from TRL, or alternatively to mask or alter the conformation of apoE on these particles. Some studies have demonstrated that the ability of apoC-I to inhibit cellular TRL uptake is greater than that of apoC-III (17, 20), although others have indicated the opposite (18, 22, 23). Irrespective of the relative capacity of C apolipoproteins to inhibit plasma clearance of TRL or their remnants, the impor-

Abbreviations: apo, apolipoprotein; CHL, combined hyperlipidemic(s); FTR, fractional transport rate; GC-MS, gas chromatography-mass spectrometry; HTG, hypertriglyceridemic(s); NL, normolipidemic(s); RT, residence time; TG, triglyceride; TR, transport rate; TRL, triglyceride-rich lipoprotein.

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tance of apoC-I in this process has been clearly demonstrated by in vivo work in apoC-I transgenic mice. Hepatic overexpression of human apoC-I results in excess apoC-I on circulating TRL, impaired uptake of TRL by the liver, plasma accumulation of VLDL and IDL particles, and elevated levels of both total triglyceride and cholesterol (24–27). These mice develop dry and scaly skin, loss of hair, and atrophic sebaceous glands lacking sebum, suggesting a role of apoC-I in lipid synthesis in the sebaceous gland and/or epidermis (28). They also have complete deficiency of subcutaneous fat, a greater than 50% reduction in abdominal adipose tissue mass, and reduced plasma non-esterified fatty acid clearance, implying an important role for apoC-I in tissue uptake of fatty acids (29) and in the etiology of obesity (30).

Although the in vivo metabolism of human apolipoproteins has been extensively investigated, very few studies have focused on apoC-I. The main reason is that apoC-I lacks tyrosine (3, 4), and it is difficult to label this protein with radioactive iodine. Conventional turnover studies have therefore been difficult to perform. Malmendier et al. overcame this problem by conjugating apoC-I to radiolabeled Bolton and Hunter reagent (BH) (31), and were able to show that the plasma residence time of apoC-I in normolipidemic volunteers was 3.2 days, and the rate of plasma apoC-I production was on average 1.8 mg/kg per day. To our knowledge, the plasma kinetics of apoC-I in hyperlipidemic subjects has not been previously investigated. In view of the role of apoC-I in receptor-mediated clearance of TRL from the circulation, we initially hypothesized that any increase in plasma VLDL concentration in hypertriglyceridemic subjects would be associated with a significant increase in VLDL apoC-I residence time. At the same time however, we have observed a significant increase in VLDL apoE and apoC-III rates of production in patients with elevated triglyceride levels (32). In order to determine whether hypertriglyceridemic subjects are characterized by decreased VLDL apoC-I catabolism or increased VLDL apoC-I production, we carried out the present study using a primed constant infusion of deuterated leucine. We have measured the endogenous rate of incorporation of labeled amino acid into VLDL and HDL apoC-I (thus circumventing the difficulty of radiolabeling this protein), and have compared the kinetic parameters of normolipidemic and hypertriglyceridemic subjects.

## METHODS

### Study subjects

A total of 14 subjects (13 males and one female) were investigated and each gave their informed consent to participate. They were part of two previous studies (32, 33) approved by the Ethics Committee of the Clinical Research Institute of Montreal involving the investigation of plasma apoE kinetics. Four subjects were normolipidemic (NL). They were apparently healthy male subjects who were selected because they had a fasting plasma triglyceride (TG) concentration <2.2 mmol/l, a total plasma cholesterol concentration <5.2 mmol/l, and were within 10% of desirable body weight. They had no evidence nor history of dyslipidemia, diabetes mellitus, nor other metabolic disorder, and were not taking medications known to affect plasma lipid metabolism. Four 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. They were classified as having type IV hyperlipoproteinemia since their LDL cholesterol (LDL-C) levels were <3.4 mmol/l. An additional six patients were also recruited from our lipid clinic who were classified as having type IIb hyperlipoproteinemia or combined hyperlipidemia (CHL) (i.e., TG > 2.2 mmol/l and LDL-C > 3.4 mmol/l). Patients taking lipid-lowering medications (statins or fibrates) were asked to stop their medications 30 days prior to their infusion experiments.

### Stable isotope infusion

After a 12-h overnight fast, study subjects were given a primed constant intravenous infusion of deuterium-labeled leucine (L-[$D_3$]leucine 98%, Cambridge Isotope Laboratories, MA), as previously described (32, 34). They were injected via a needle attached to a left forearm vein with 10 μmol per kg body weight of L-[$D_3$]leucine, dissolved in physiological saline, followed by a 12-h constant infusion (given by peristaltic pump) of 10 μmol L-[$D_3$] leucine/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 and 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. Lipoproteins were recovered in the supernate by tube-slicing. ApoC-I was separated from other apolipoproteins in VLDL and HDL by preparative electrophoresis using polyacrylamide-urea (8 M, pH 9.1) gels (35), as shown in **Fig. 1**. IDL/LDL apoC-I was also separated by electrophoresis, but in a large number of cases insufficient apoC-I was obtained for analysis of leucine enrichment. It is possible that this was due to the large amount of apoB-100 in IDL/LDL, which interfered with the recovery of soluble proteins during sample delipidation before electrophoresis. Coumassie blue staining was used to identify the position of apolipoproteins in polyacrylamide gels.

### 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 apoB and apoA-I concentrations were measured by nephelometry on a Behring Nephelometer 100 (Behring) using Behring protocol and reagents. Plasma and lipoprotein apoC-I concentrations were measured with an ELISA developed in our laboratory. An immunopurified polyclonal goat anti-human apoC-I antibody was used as both capture and detection antibody (Biodesign, Kennebunk, ME). The apoC-I assay was calibrated with standard plasmas kindly provided by Dr. Petar Alaupovic (Oklahoma Medical Research Foundation, Oklahoma City). The intraassay and interassay CVs were 2.2% and 9.8%, respectively. Total recovery (mean ± SD) of apoC-I in lipoprotein

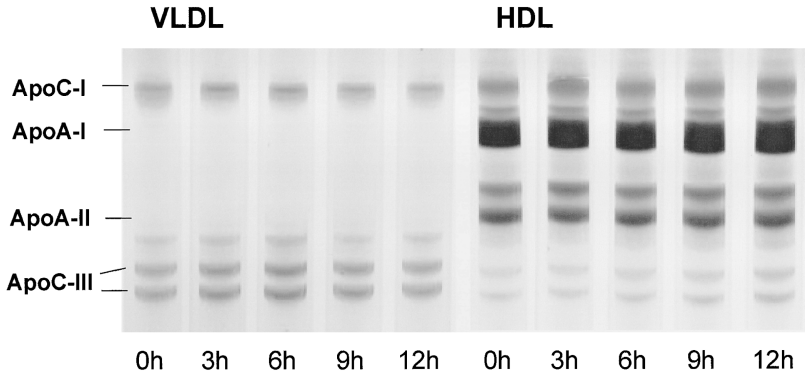


**Fig. 1.** Separation of VLDL and HDL apoC-I by 8 M urea polyacrylamide gel electrophoresis (pH 9.1). Gels are shown for lipoprotein fractions isolated 0, 3, 6, 9, and 12 h after the start of the stable isotope infusion. Apolipoprotein bands are indicated on the left of the figure.

fractions separated by ultracentrifugation was between 70% and 80%. Lipoprotein apoC-I levels were corrected proportionally to give 100% recovery. ApoC-I in the bottom fractions, which represented 3% or less of total plasma apoC-I, was considered to be part of the HDL apoC-I kinetic pool.

### Determination of isotopic enrichment

ApoC-I bands, as well as blank (non-protein containing) gel slices were excised from polyacrylamide gels. 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 heptaflurobutyric anhydride (Supelco) for 20 min at 60°C (34). 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, 5,988 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. Tracer to tracee ratios were corrected for background leucine in gel slices (and for leucine introduced during the amino acid purification and derivitization procedures) by estimating the amount of leucine in processed blank gel slices in relation to the norleucine internal standard. Background leucine represented 10% or less of total leucine recovered in apoC-I samples.

### Kinetic analysis

Plasma leucine enrichment at plateau (measured eight times during the course of the infusion experiment) was used as a measure of precursor pool enrichment, which was assumed to remain constant throughout the infusion. Although plateau enrichment of VLDL apoB is a more accurate measure of leucine enrichment of the hepatic precursor pool (34), it was not used in the present study, since hypertriglyceridemic patients with increased VLDL apoB pool sizes often do not achieve a VLDL apoB plateau during a 12-h infusion. Use of plasma leucine enrichment leads to an underestimation of fractional synthetic rates by 10–20%, but it was assumed that this underestimation was the same for the normal and hyperlipidemic subjects. Mean (± SE) plasma leucine tracer to tracee ratio for the 14 individuals combined was 10.02 ± 0.64%.

Stable isotope enrichment of VLDL and HDL apoC-I, expressed as a tracer to tracee ratio, was plotted against time. SAAM II computer software (SAAM II institute, WA) and a three compartment model were used to analyze enrichment curves, where the first compartment represented the plasma amino acid precursor pool and the second compartment was a delay compartment, accounting for the synthesis, assembly, and secretion of apolipoprotein. The third compartment was the plasma protein compartment. The slope of apoC-I enrichment curves (relative to plasma leucine at plateau) was indicative of the overall fractional rate of appearance of apoC-I into VLDL and HDL. These pools were treated as if they were single homogeneous entities, however, metabolically they were probably made up of several different metabolic "sub-pools" of apoC-I, which could have been slow- or fast-turning over pools, transferrable- or non-transferrable, exchangeable or non-exchangeable. We have assumed that all these metabolic pools were accessible to physiological labeling during the 12-h infusion period, while subjects remained in the fasted state. Fractional rate of appearance (i.e., fractional transport rate, FTR) of apoC-I into VLDL or HDL thus represented the mean weighted average of fractional appearance rates into different apoC-I sub-pools. Calculated residence times (RT) (the reciprocal of FTR) were thus also mean weighted average residence times for apoC-I in different sub-pools. They were indicative of the average time that an apoC-I molecule was present in VLDL or HDL, irrespective of whether it was transformed into the apolipoprotein of another lipoprotein, exchanged to another lipoprotein, or catabolized by a specific tissue. TRs were calculated (in mg/kg.day) as:

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

where: pool size = plasma concentration (mg/dl) × plasma volume (0.045 l/kg). TR was also expressed in molar units (nmol/kg.day) using a molecular weight of 6,613 Da for apoC-I.

### Statistical analysis

Statistical significance of differences between mean values was assessed by unpaired Student's $t$-tests using SigmaStat software (Jandel Scientific, CA). Pearson correlation coefficients ($r$) were calculated to describe the correlation between different kinetic and mass parameters.

TABLE 1. Characteristics of study subjects

| | Normolipidemics (n = 4) | Hypertriglyceridemics (n = 4) | Combined Hyperlipidemics (n = 6) |
|---|---|---|---|
| Age (years) | 30 ± 2 | 52 ± 8[a] | 47 ± 6 |
| BMI (kg/m$^2$) | 24.6 ± 0.5 | 26.2 ± 1.2 | 26.8 ± 0.9 |
| Plasma triglyceride (mmol/l) | 0.72 ± 0.03 | 4.89 ± 0.72[b] | 2.56 ± 0.40[b] |
| Plasma cholesterol (mmol/l) | 3.82 ± 0.34 | 5.08 ± 0.72 | 7.46 ± 0.26[c] |
| VLDL triglyceride (mmol/l) | 0.59 ± 0.03 | 4.32 ± 0.77[b] | 2.20 ± 0.39[a] |
| VLDL cholesterol (mmol/l) | 0.18 ± 0.02 | 1.98 ± 0.64[a] | 1.03 ± 0.22[a] |
| LDL cholesterol (mmol/l) | 2.34 ± 0.22 | 2.48 ± 0.26 | 5.35 ± 0.48[b] |
| HDL cholesterol (mmol/l) | 1.35 ± 0.16 | 0.62 ± 0.05[b] | 1.08 ± 0.11 |
| ApoB (mg/dl) | 75 ± 5 | 126 ± 9[b] | 193 ± 11[c] |
| ApoA-I (mg/dl) | 130 ± 11 | 116 ± 6 | 113 ± 6 |

Values represent means ± SE. Measurements were made on fasting plasma obtained at the screening visit. Significantly different from normolipidemic subjects by unpaired *t*-test:
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

## RESULTS

### Characteristics of study subjects

Plasma lipid and lipoprotein characteristics of study subjects are shown in **Table 1**. HTG and CHL patients tended to be older, though their mean BMIs were not significantly different relative to NL subjects. HTG and CHL patients had significantly increased plasma triglyceride levels compared with NL, as well as significantly increased VLDL triglyceride and cholesterol levels. Mean plasma concentrations of VLDL apoB in NL, HTG, and CHL were 4.2 ± 0.5, 26.1 ± 5.4, and 15.5 ± 2.3 mg/dl, respectively. Thus, plasma VLDL levels were on average 2-fold higher in HTG versus CHL patients. According to selection criteria, CHL patients also had significantly increased levels of plasma cholesterol, LDL-C and apoB. HTG and CHL patients tended to have lower levels of HDL-C and apoA-I, however, this was statistically significant only for HDL-C concentration in HTG patients.

### Plasma and lipoprotein apoC-I levels

HTG and CHL patients had significantly elevated levels of total plasma apoC-I, which were on average 50% higher than those of NL subjects (**Table 2**). Increased levels of apoC-I were predominantly due to increased levels of VLDL apoC-I, although HTG and CHL patients also had significantly increased levels of apoC-I in their IDL/LDL fraction relative to NL. HDL apoC-I concentrations tended to be lower in HTG and CHL patients, but this was not statistically significant. On average, 92% of total plasma apoC-I was found in HDL in NL compared with 46% in HTG and 49% in CHL patients. Less than 3% of total plasma apoC-I was recovered in the d > 1.21 g/ml fraction after ultracentrifugation.

### Incorporation of labeled leucine into VLDL and HDL apoC-I

Deuterated leucine was detected in VLDL and HDL apoC-I in all subjects 1 to 2 h after the start of the amino

TABLE 2. Plasma concentration of apoC-I in lipoprotein fractions isolated by ultracentrifugation

| | Normolipidemics (n = 4) | Hypertriglyceridemics (n = 4) | Combined Hyperlipidemics (n = 6) |
|---|---|---|---|
| VLDL | 0.3 ± 0.1 | 5.8 ± 0.8[c] | 4.5 ± 0.8[b] |
| | (4.1 ± 1.2%)[d] | (46.3 ± 4.1%) | (35.4 ± 3.3%) |
| IDL+LDL | 0.2 ± 0.1 | 0.8 ± 0.2[b] | 1.7 ± 0.2[c] |
| | (2.2 ± 0.4%) | (6.0 ± 0.9%) | (14.1 ± 1.9%) |
| HDL | 7.3 ± 0.5 | 5.7 ± 0.6 | 6.0 ± 0.7 |
| | (92.2 ± 1.7%) | (45.5 ± 3.4%) | (48.7 ± 3.6%) |
| d > 1.21 g/ml | 0.1 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0.1 |
| | (1.6 ± 0.5%) | (2.2 ± 0.7%) | (1.8 ± 0.5%) |
| Total | 7.9 ± 0.6 | 12.5 ± 1.2[a] | 12.4 ± 1.3[a] |

Concentrations (mg/dl) represent means ± SE for 'n' individuals in each group. Five measurements made at 3-h intervals during the stable isotope infusion were averaged to give individual plasma and lipoprotein apoC-I concentrations.
Significantly different from normolipidemic subjects by unpaired Student's *t*-test:
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] Percentages in parentheses represent the relative amount of total plasma apoC-I in each lipoprotein fraction.

acid infusion, as depicted in **Fig. 2**. Rate of appearance of stable isotope-labeled apoC-I in both VLDL and HDL was linear during the 12-h time course of the experiment. In all subjects, the VLDL and HDL apoC-I leucine incorporation curves were distinct (i.e., they were not superimposable), and without exception, rate of appearance of apoC-I containing deuterated leucine was significantly greater in VLDL compared with HDL. The distinct nature of the VLDL and HDL apoC-I enrichment curves indicated that not all apoC-I was freely exchangeable between these two pools (i.e., a significant proportion of VLDL apoC-I was metabolically distinct from HDL apoC-I, and a significant proportion of HDL apoC-I was metabolically distinct from VLDL apoC-I).

### VLDL and HDL apoC-I kinetics

Kinetic parameters were derived for all 14 subjects and mean data are shown in **Table 3**. In NL subjects, mean VLDL apoC-I transport rate was 0.24 ± 0.11 mg/kg.day, which in molar terms was 35.5 ± 17.2 nmol/kg.day (mol wt. apoC-I = 6,613 Da). Residence time of VLDL apoC-I in circulating blood was 0.74 ± 0.17 days, or in other words, each molecule of apoC-I remained in this VLDL fraction for an average of 17.8 h. In comparison, apoC-I remained in the HDL fraction of NL for an average of 3.6 days. Rate of transport of HDL apoC-I in NL was on average 4-fold greater than VLDL apoC-I transport (i.e., 0.96 ± 0.12 mg/kg.day or 144.4 ± 17.7 nmol/kg.day). Significantly elevated levels of VLDL apoC-I in HTG and CHL patients were associated with 10- to 15-fold increases in VLDL apoC-I transport. In molar terms, VLDL apoC-I transport was 347 ± 52 and 460 ± 80 nmol/kg.day in HTG and CHL, respectively. HTG and CHL VLDL apoC-I residence times were not significantly different from those of NL subjects. Neither apoC-I TR or RT were significantly related to LDL-C concentrations (all subjects combined, n = 14). HDL apoC-I residence times were significantly higher (4- to 5-fold) than VLDL apoC-I residence times in HTG and CHL, as well as in NL. Although HDL apoC-I transport rates were lower and residence times were higher than NL in HTG patients, this was not the case in CHL (Table 3). For the 14 subjects combined, HDL apoC-I TR was inversely related to plasma triglyceride ($r = -0.63$, $P < 0.05$) and also VLDL triglyceride concentration ($r = -0.62$, $P < 0.05$). No significant correlations were observed, however, between HDL apoC-I levels and either HDL-C or apoA-I levels, and similarly there were no significant correlations between apoC-I kinetic parameters and plasma HDL concentration.

## DISCUSSION

The present results show that patients with elevated levels of total plasma and VLDL triglyceride have increased levels of VLDL apoC-I, which are associated with increased rates of VLDL apoC-I transport. VLDL apoC-I residence time was not increased in these patients, even in those that had increased levels of plasma LDL-C. HDL apoC-I levels on the other hand were unchanged or tended to be lower in hypertriglyceridemic patients compared with controls, and this was associated with a decrease in rates of HDL apoC-I transport.

A likely explanation for increased levels of VLDL apoC-I transport (i.e., production) in patients with increased levels of plasma VLDL is that increased triglyceride secretion and/or increased VLDL production by the liver is able to directly stimulate hepatic apoC-I synthesis and secretion. Presumably, increased amounts of VLDL apoC-I are necessary for the normal metabolic processing of these particles. No in vitro data using isolated tissues or cultured cells has been published, however, in order to substantiate the fact that stimulation of hepatic VLDL production leads to increased apoC-I synthesis and/or secretion. An alternative explanation is that increased hepatic apoC-I production might, in some individuals, be the precursor of increased hepatic triglyceride or VLDL secretion. This is unlikely however, based on results from mice

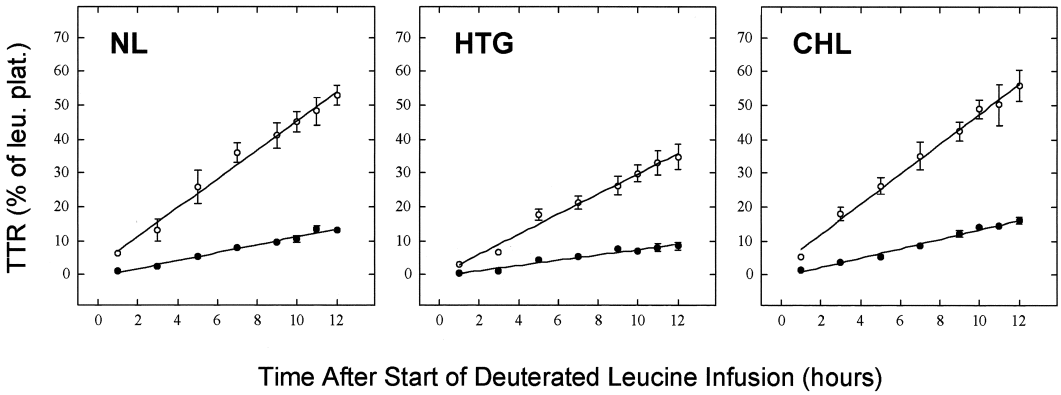


**Fig. 2.** Enrichment of VLDL (open symbols) and HDL (closed symbols) apoC-I with deuterated leucine in normolipidemic (NL), hypertriglyceridemic (HTG), and combined hyperlipidemic (CHL) subjects. Values represent means ± SEM. For each individual, VLDL and HDL apoC-I tracer to tracee ratios were expressed as a percentage relative to plateau enrichment of plasma leucine in order to normalize data and allow results from different individuals to be combined.

TABLE 3. VLDL and HDL apoC-I kinetic parameters in different patient groups

| | VLDL ApoC-I | | | HDL ApoC-I | | |
|---|---|---|---|---|---|---|
| | TR | RT | Pool Size | TR | RT | Pool Size |
| | *mg/kg.day* | *days* | *mg* | *mg/kg.day* | *days* | *mg* |
| Normolipidemics | | | | | | |
| 1 | 0.07 | 0.50 | 2.8 | 0.82 | 3.79 | 240.9 |
| 2 | 0.13 | 0.80 | 7.5 | 0.73 | 3.86 | 201.9 |
| 3 | 0.57 | 0.47 | 20.1 | 1.26 | 2.87 | 272.8 |
| 4 | 0.17 | 1.20 | 16.7 | 1.01 | 3.75 | 311.1 |
| Mean ± SE | 0.24 ± 0.11 | 0.74 ± 0.17 | 11.7 ± 4.0 | 0.96 ± 0.12 | 3.57 ± 0.23 | 256.7 ± 23.2 |
| Hypertriglyceridemics | | | | | | |
| 1 | 2.44 | 1.46 | 259.8 | 0.53 | 5.14 | 200.1 |
| 2 | 1.72 | 1.21 | 135.4 | 0.71 | 4.96 | 226.5 |
| 3 | 3.20 | 0.88 | 193.4 | 0.37 | 6.51 | 167.7 |
| 4 | 1.81 | 1.08 | 158.6 | 0.49 | 4.09 | 162.2 |
| Mean ± SE | 2.29 ± 0.34[b] | 1.16 ± 0.12 | 186.8 ± 27.1[c] | 0.53 ± 0.07[a] | 5.18 ± 0.50[a] | 189.1 ± 15.0 |
| Combined hyperlipidemics | | | | | | |
| 1 | 2.11 | 0.61 | 94.2 | 1.20 | 2.10 | 186.1 |
| 2 | 3.47 | 0.48 | 139.9 | 1.52 | 2.69 | 344.0 |
| 3 | 3.81 | 0.66 | 205.0 | 1.17 | 2.65 | 254.9 |
| 4 | 4.99 | 0.73 | 327.0 | 0.84 | 3.56 | 266.6 |
| 5 | 2.45 | 0.72 | 159.9 | 0.94 | 2.12 | 180.2 |
| 6 | 1.43 | 0.96 | 109.2 | 0.61 | 3.26 | 157.6 |
| Mean ± SE | 3.04 ± 0.53[b] | 0.69 ± 0.06 | 172.5 ± 34.8[b] | 1.05 ± 0.13 | 2.73 ± 0.24 | 231.6± 28.6 |

Values are for individual patients. TR, transport rate; RT, residence time.
Statistically different from normolipidemics by unpaired Student's *t*-test:
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

overexpressing human apoC-I, in which there is no evidence of increased hepatic triglyceride or VLDL production (25–27). In addition, apoC-I-deficient mice are not hypotriglyceridemic, nor do they appear to have an impairment in hepatic VLDL production (36, 37).

The foregoing discussion assumes that VLDL apoC-I transport, as measured with the current methodology, represents direct tissue output of apoC-I bound to VLDL. It cannot be overlooked, however, that apoC-I is an exchangeable apolipoprotein, which not only can transfer spontaneously between VLDL and HDL, but can also be metabolically converted from VLDL apoC-I to HDL apoC-I, or vice versa (1, 2). Our data show that not all VLDL apoC-I is metabolically interchangeable with HDL apoC-I, as evidenced by the distinct VLDL and HDL apoC-I enrichment curves in Fig. 2. At the same time however, it is not possible to accurately determine what proportion of apoC-I transport into VLDL is due to direct tissue production of VLDL apoC-I. By monitoring the overall fractional rate of transport of apoC-I into VLDL and HDL, we have calculated a residence time for apoC-I, which was indicative of the average time that an apoC-I molecule was present in VLDL or HDL, irrespective of whether it was transformed into the apolipoprotein of another lipoprotein, exchanged to another lipoprotein, or catabolized by a specific tissue. From this residence time, we have determined a transport rate which reflected the mean weighted flux of apoC-I moving in and out of this pool per day, again irrespective of whether it came directly from tissue or indirectly from another lipoprotein in plasma. Therefore, in the absence of data concerning apoC-I conversion or exchange, it remains a possibility that increased VLDL apoC-I transport in patients with elevated triglyceride levels represents, at least in part, increased conversion/exchange of HDL apoC-I to VLDL apoC-I.

As mentioned earlier, only one other study has previously investigated apoC-I kinetics in humans. Malmendier et al. demonstrated that mean plasma apoC-I production in four normolipidemic male subjects was 1.8 mg/kg per day and plasma apoC-I residence time was 3.2 days (31). More than 80% of injected $^{125}$I-BH-apoC-I was associated with plasma HDL in this study, and indeed these parameters are comparable with our HDL apoC-I kinetic parameters for NL, i.e., 0.96 mg/kg.day and 3.6 days, respectively. A unique feature of the present study, however, is that we have also been able to derive kinetic parameters for VLDL apoC-I, and it is interesting that in normolipidemic subjects, HDL apoC-I transport was about 4-times greater than that of VLDL apoC-I transport, whereas in hypertriglyceridemics VLDL apoC-I transport was 2- to 6-times greater than that of HDL. In molar terms, VLDL apoC-I transport was on average 400 nmol/kg.day in hypertriglyceridemic subjects, which was greater than that of VLDL apoE (~130 nmol/kg.day), but less than that of VLDL apoC-III (~600 nmol/kg.day) in these individuals (32). VLDL apoC-I transport (~35 nmol/kg.day) was also less than VLDL apoC-III transport (~150 nmol/kg.day) in normolipidemic subjects, but was similar in magnitude to that of VLDL apoE (~45 nmol/kg.day) (32). These data suggest that in both normolipidemic and hypertriglyceridemic subjects, more molecules of VLDL apoC-III are transported per day than molecules of VLDL apoE or

VLDL apoC-I. At the same time, however, hypertriglyceridemia results in a greater increase of VLDL apoC-I transport compared with that of VLDL apoE or VLDL apoC-III (i.e., hypertriglyceridemics have 10-fold higher VLDL apoC-I, 3-fold higher VLDL apoE and 4-fold higher VLDL apoC-III transport rates relative to normolipidemics).

In carrying out the present study, we initially hypothesized that any increase in plasma VLDL concentration in hypertriglyceridemic subjects would be associated with a significant increase in VLDL apoC-I residence time. Our hypothesis was based on considerable evidence showing that apoC-I plays an important role in regulating receptor-mediated uptake of TRL (17–23). We thought that an increase in VLDL apoC-I residence time would be particularly evident in patients with increased LDL-C levels and reduced LDL-receptor activity. No significant relationship was found, however, between VLDL apoC-I kinetic parameters and LDL-C, and there was no significant correlation between VLDL apoC-I residence times and VLDL triglyceride, VLDL-C or VLDL apoB levels. These data do not refute that apoC-I may be having a significant impact on plasma TRL catabolism in vivo. They do, however, point out that residence time of apoC-I in VLDL is not a critical factor in determining plasma TRL levels. It is interesting to note that the average residence time of VLDL apoB in normolipidemic male subjects is 0.12 days or 2.9 h (38), whereas the plasma residence time of VLDL apoC-I is 0.74 days or 18 h (Table 3). This suggests that apoC-I is associated with a VLDL subfraction that has an unusually long plasma residence time. Alternatively, apoC-I exchanges between VLDL particles and does not remain attached to a particular VLDL particle as it is lipolytically converted to IDL/LDL or is cleared from the blood circulation by a given tissue.

It is interesting that recent work by Gautier et al. (16) has provided convincing evidence that apoC-I is the protein in HDL which accounts for the CETP-inhibitory activity that is specifically associated with human HDL. This suggests that apoC-I may be very important in determining plasma HDL-C levels, particularly when it is associated with HDL. In the present study, no significant correlation was apparent between HDL apoC-I levels and either HDL-C or apoA-I levels, and somewhat surprisingly, we found no significant relationship between HDL apoC-I kinetic parameters and plasma HDL-C concentration. HDL apoC-I rates of transport were nevertheless inversely related to plasma and VLDL-TG levels. It must be remembered that the present subjects were selected primarily on the basis of their plasma triglyceride and LDL-C levels, and they did not have a large variation in HDL apoC-I levels, which may be an explanation for the lack of significant correlations with this latter parameter.

In conclusion, the present study has shown that hypertriglyceridemic patients (with or without elevated LDL-C levels) have increased plasma concentrations of VLDL apoC-I and increased levels of VLDL apoC-I transport. These results suggest that hepatic VLDL apoC-I production is increased in hypertriglyceridemic subjects. Although they did not have significantly reduced HDL apoC-I levels, hypertriglyceridemic subjects also tended to have reduced levels of HDL apoC-I transport. Since recent evidence suggests that inhibition of CETP activity may be antiatherogenic, and that apoC-I appears to play an important role in inhibiting CETP, additional studies are warranted to define factors affecting plasma lipoprotein apoC-I concentration and distribution.

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