Apolipoprotein C-III and the Metabolic Basis for Hypertriglyceridemia and the Dense Low-Density Lipoprotein Phenotype

Chunyu Zheng, ScD; Christina Khoo, PhD; Jeremy Furtado, ScD; Frank M. Sacks, MD

**Background**—Here, we aim to identify defects of apolipoprotein (apo) B lipoprotein metabolism that characterize hypertriglyceridemia, focusing on apoC-III and apoE.

**Methods and Results**—We studied the transport of plasma apoB within 21 distinct subfractions as separated by anti–apoC-III and anti–apoE immunoaffinity chromatography and ultracentrifugation in 9 patients with moderate hypertriglyceridemia and 12 normotriglyceridemic control subjects. Hypertriglyceridemia was characterized by a 3-fold higher liver secretion of very low-density lipoprotein (VLDL) that had apoC-III but not apoE and a 50% lower secretion of VLDL with both apoC-III and apoE (both \( P < 0.05 \)). This shift in VLDL secretion pattern from apoE to apoC-III resulted in significantly reduced clearance of light VLDL (−39%; \( P < 0.05 \)), compatible with the antagonizing effects of apoC-III on apoE-induced clearance of triglyceride-rich lipoproteins. In addition, rate constants for clearance were reduced for apoE-containing triglyceride-rich lipoproteins in hypertriglyceridemia, associated with increased apoC-III contents of these particles. LDL distribution shifted from light and medium LDL to dense LDL in hypertriglyceridemia through a quartet of kinetic perturbations: increased flux from apoC-III–containing triglyceride-rich lipoproteins, a shift in liver LDL secretion pattern from light to dense LDL, an increased conversion rate from light and medium LDL to dense LDL, and retarded catabolism of dense LDL.

**Conclusions**—These results support a central role for apoC-III in metabolic defects leading to hypertriglyceridemia. Triglyceride-rich lipoprotein metabolism shifts from an apoE-dominated system in normotriglyceridemic participants characterized by rapid clearance from circulation of VLDL to an apoC-III–dominated system in hypertriglyceridemic patients characterized by reduced clearance of triglyceride-rich lipoproteins and the formation of the dense LDL phenotype. *(Circulation. 2010;121:1722-1734.)*

**Key Words:** apolipoproteins ■ lipids ■ lipoproteins ■ metabolism

Hypertriglyceridemia is a common form of dyslipidemia that affects 30% of adults in the United States and is frequently associated with increased risk for coronary heart disease (CHD). However, unlike the relationship between hypercholesterolemia and CHD, which has been firmly established by observational studies, pathophysiology, and clinical trials, the direct involvement of plasma triglyceride in atherosclerosis and the subsequent development of CHD has been more difficult to prove. Elevated triglyceride levels are thought to increase CHD risk through the atherogenic effects of triglyceride-rich lipoproteins (TRLs) and their remnants. However, there is substantial heterogeneity in TRLs; triglyceride can be packaged into a wide variety of lipoprotein particles with potentially varying degrees of atherogenicity. Routine lipoprotein analyses do not identify these particles, so the atherogenic potential of hypertriglyceridemic states may not be adequately appreciated.

**Clinical Perspective on p 1734**

Apolipoproteins have long been suspected to play crucial roles in the origin of hypertriglyceridemia in humans. Among them, apolipoprotein (apo) C-III, a small, surface protein found on many TRLs, has been proposed as a key contributor to hypertriglyceridemia on the basis of its inhibitory effects on apoB lipoprotein catabolism. In vitro experiments have demonstrated that apoC-III inhibits lipoprotein lipase and hepatic lipase and retards clearance of very low-density lipoprotein (VLDL) by interfering with binding of apoB100 or apoE to hepatic receptors. Overexpression of apoC-III in mice causes hypertriglyceridemia; apoC-III deficiency pro-

Received April 25, 2009; accepted February 17, 2010.
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Guest Editor for this article was Donna K. Arnett, PhD.
The online-only Data Supplement is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.109.875807/DC1.
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*Circulation* is available at http://circ.ahajournals.org DOI: 10.1161/CIRCULATIONAHA.109.875807

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tects against it. In humans, kinetic studies suggest that apoC-III bound to TRL correlates positively with the secretion of VLDL triglyceride and negatively with VLDL catabolism. In addition, a recent genome-wide association study identifies apoC-III as the major determinant of fasting and postprandial triglyceride levels. On the other hand, overexpression of apoE in mice corrects the hypertriglyceridemia produced by overexpression of apoC-III. However, it is less clear how closely these findings from in vitro experiments and animal studies map to TRL metabolism and defective composition of TRL.

Previously, we reported that a major metabolic defect of hypertriglyceridemic patients is the selective overproduction of a subtype of VLDL containing apoC-III but not apoE and a global reduction in the clearance rate from plasma of all TRLs. The
present kinetic studies extend these findings to a significantly larger sample to develop a more highly powered kinetic model of plasma apoB lipoprotein metabolic pathways that establish hypertriglyceridemia. We also studied pathways that establish the dense LDL phenotype in hypertriglyceridemia. Our findings support the fundamental involvement of apoC-III as a component of VLDL and intermediate-density lipoprotein (IDL) in abnormal metabolism causing hypertriglyceridemia and the protective role of apoE underlying normal metabolism of apoB lipoproteins. Our findings also reveal 4 major metabolic processes that give rise to the dense LDL phenotype in hypertriglyceridemia, including channeling of apoC-III-containing TRL to LDL, a shift in hepatic secretion of LDL from light to dense particles, increased conversion in plasma of light to dense LDL, and slow clearance of dense LDL.

Methods
A detailed Methods section is available in the online-only Data Supplement. Briefly, 9 patients with moderate hypertriglyceridemia and 12 normotriglyceridemic control subjects participated in the tracer kinetics studies after 3 weeks on a controlled high-carbohydrate, low-fat diet. This type of diet was used because it promotes dense LDL. Participants received a 15-hour primed, continuous infusion of [D3]L-leucine and a bolus injection of [D5]L-phenylalanine. Blood samples were collected at baseline, every 20 minutes in the first 2 hours of infusion, and hourly thereafter.

ApoB lipoproteins from these samples were separated by anti-apoC-III and anti-apoE immunoaffinity columns into those containing both apolipoproteins (E+CIII+), either one (E+CIII, E−CIII+), or none of them (E−CIII−). These 4 immunofractions were further separated according to density into 6 fractions: light LDL (LDL-1), medium LDL (LDL-2), and dense LDL (LDL-3). Thus, 24 distinct apoB lipoprotein fractions were obtained for each sample. ApoB was precipitated from these fractions, and tracer enrichment and tracee mass were measured by gas chromatography–mass spectrometry. A multicompartment model was used to find the best fit to the observed data with SAAM II software (Figure 2). For each individual, both [D3]L-leucine and [D5]L-phenylalanine tracer data were included in the same model, and the data were solved simultaneously by making the rate constants equal for the leucine and phenylalanine tracer experiments. Thus, for each participant, a single set of rate constants was produced. This model was able to generate excellent fits to the tracer data (Figure 1) and to the apoB pool sizes.

Results
Nine hypertriglyceridemic patients and 12 age- and gender-matched normotriglyceridemic control subjects participated in the study. At the screening visit when the participants were eating their usual diet, triglyceride levels were 286±15 mg/dL for hypertriglyceridemic patients versus 93±18 mg/dL for normotriglyceridemic control subjects (*P<0.01). Subject characteristics and concentrations of plasma lipids and apolipoproteins after the 3-week controlled low-fat diet are summarized in Tables 1 and 2.

ApoB lipoproteins were separated into 21 compartments by density and apolipoprotein composition. Each plasma apoB compartment represents a physically isolated lipoprotein fraction. See the Methods section in the online-only Data Supplement for model development and validation. LDL-1 indicates light LDL; LDL-2, medium LDL; LDL-3, dense LDL.
Although all subjects were nondiabetic and had normal glucose levels and homeostasis model assessment (HOMA) index, hypertriglyceridemic patients had significantly higher glucose, insulin, HOMA index, and body mass index than normotriglyceridemic control subjects. Hypertriglyceridemic patients also had significantly higher plasma apoC-III and apoE, as well as VLDL apoB, apoC-III, and apoE. The plasma total apoC-III concentration in hypertriglyceridemic patients was double that in normotriglyceridemic control subjects, whereas apoE was higher by only about one-third. In addition, hypertriglyceridemic patients had lower light and medium LDL and higher dense LDL levels, reflecting a dense LDL phenotype.

**Accumulation of E–CIII+ TRL in Hypertriglyceridemia**

Compared with control subjects, hypertriglyceridemic patients had significantly higher pool sizes of light VLDL, dense VLDL, and IDL that had apoC-III but not apoE (E–CIII+) (Figure 3). E–CIII+ light VLDL pool size was 3.4-fold higher in hypertriglyceridemic patients (57.0±11.7

![Figure 3. Plasma apoB pool sizes. Open bars indicate apoB pool sizes among normotriglyceridemic control subjects (NTG); solid bars, hypertriglyceridemic patients (HTG). Data represent mean±SEM (n=12 for NTG, n=9 for HTG). The numbers on the bottom right represent apoB pool sizes in E–CIII+ LDL-3. *P<0.05 between NTG and HTG by unpaired t test.](http://circ.ahajournals.org/lookup/doi/10.1161/CIRCULATIONAHA.117.027254)
versus 16.8±4.5; *P<0.05). In contrast, the 2 groups had similar pool sizes of VLDL and IDL that had apoE (E+CIII−, E+CIII+). Hypertriglyceridemic patients also had higher pool sizes of E−CIII− VLDL.

**Increased Liver Secretion of E−CIII+ Light VLDL in Hypertriglyceridemia**
Accumulation of E−CIII+ TRL was due at least in part to the fact that hypertriglyceridemic patients had 3-fold higher secretion rates of E−CIII+ light VLDL and 40% lower secretion of E+CIII+ light VLDL (Figure 4). Hypertriglyceridemic patients also had higher secretion of light E−CIII− VLDL. Overall, hypertriglyceridemic patients had lower secretion rates of apoE-containing VLDL and IDL. Total apoB secretion rates were similar between hypertriglyceridemic patients and normotriglyceridemic control subjects (23.7±1.4 versus 26.3±1.5 mg·d·kg−1; *P=0.56).

**Slow Metabolism of ApoE-Containing VLDL in Hypertriglyceridemia**
Leucine enrichment curves showed slower rates of appearances for apoE-containing VLDL and LDL among hypertriglyceridemic participants (Figure 1). Indeed, modeling results demonstrated that hypertriglyceridemic patients had significantly lower fractional catabolic rates (FCRs) for apoE-containing VLDL (both E+CIII+ and E+CIII−; Figure 5). The FCR of E+CIII− IDL was also significantly lower in hypertriglyceridemic patients. On the other hand, FCRs of VLDL and IDL without apoE, either E−CIII− or E−CIII+, were similar between the 2 groups.

**Hypertriglyceridemia Shifts ApoB Flux From TRL Containing ApoE to Those Without ApoE, Reducing VLDL Clearance and Increasing Synthesis of LDL**
As a result of higher secretion rates of E−CIII+ light VLDL (Figure 4), hypertriglyceridemic patients had significantly higher synthetic rates for its products, E−CIII− dense VLDL and IDL, lipoproteins that almost exclusively underwent further lipolytic processing to IDL and LDL, most of it ending as E−CIII− dense LDL (Figure 6A and Table I of the online-only Data Supplement). In both hypertriglyceridemic patients and normotriglyceridemic control subjects, <7% of E−CIII− VLDL and IDL were removed from circulation before they were converted to LDL. In addition, >80% of E−CIII+ LDL were converted to E−CIII− LDL. Similarly, hypertriglyceridemic patients also had higher secretion of E−CIII− VLDL, and this was processed in plasma to IDL and LDL. For both hypertriglyceridemic patients and normotriglyceridemic control subjects, the majority of E−CIII− VLDL and IDL (73% in hypertriglyceridemic patients, 90% in normotriglyceridemic control subjects; *P=0.43) were hydrolyzed into E−CIII− LDL. In contrast, hypertriglyceridemic patients had much lower secretion of E+CIII+ light VLDL and IDL, resulting in much lower flux of VLDL and IDL out of circulation.

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![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Direct apoB secretion rate. Data represent direct secretion rates into plasma (mg·d·kg−1, mean±SEM) of apoB lipoproteins according to density and apoC-III and apoE content. NTG indicates normotriglyceridemic control subjects; HTG, hypertriglyceridemic patients; LDL-1, light LDL; LDL-2, medium LDL; LDL-3, dense LDL. *P<0.05 by unpaired t test.
plasma (Figure 6A). Flux of E+CIII−TRL, a minor fraction coming from E+CIII+TRL, was also similarly reduced among hypertriglyceridemic patients. These changes among hypertriglyceridemic patients resulted in significantly reduced flux rates from faster to slower metabolized types of lipoproteins resulting in significantly reduced flux rates for clearance of light VLDL (2.27±0.40 versus 3.73±0.60 mg·d⁻¹·kg⁻¹; P<0.05) and of total VLDL (5.73±0.70 versus 7.94±0.7 mg·d⁻¹·kg⁻¹; P<0.05) compared with normotriglyceridemic control subjects.

Shift of LDL Distribution From Light and Medium LDL to Dense LDL in Hypertriglyceridemic Patients
Although total LDL pool sizes were not significantly different between hypertriglyceridemic patients and normotriglyceridemic control subjects (1914±238 versus 1710±205 mg; P=0.52), there was a shift in LDL distribution pattern (Figure 3). In all 3 LDL density subfractions, the predominant type of LDL in both groups by far was E−CIII−, making up >90% of total LDL, and hypertriglyceridemic patients had smaller pool sizes of light and medium LDL (E−CIII− LDL-1, LDL-2) but a larger pool size of dense LDL (E−CIII− LDL-3).

Shift of LDL Secretion Pattern From Light to Dense LDL in Hypertriglyceridemia
Hypertriglyceridemic patients had significantly higher secretion rates of E−CIII− LDL-3, their predominant type (1.11±0.32 versus 0.72±0.26 mg·d⁻¹·kg⁻¹; P<0.05; Figure 4). In contrast, hypertriglyceridemic patients had much lower secretion of E−CIII− LDL-1 (0.34±0.12 versus 1.03±0.42 mg·d⁻¹·kg⁻¹; P<0.05) and a trend for lower secretion of E−CIII− LDL-2. Liver secretion of LDL-3 E−CIII+ was also higher in hypertriglyceridemic patients.

Accelerated Conversion of Lighter to Denser LDL and Slow Metabolism of Dense LDL in Hypertriglyceridemia
Rate constants for conversion of lighter LDL to denser LDL (LDL-1 to LDL-2, LDL-2 to LDL-3) were significantly higher in hypertriglyceridemic patients for E−CIII−, E−CIII+, and E+CIII+ (Figure 6B). On the other hand, hypertriglyceridemic patients had significantly slower FCRs of E−CIII− LDL-3 (0.75±0.13 versus 1.17±0.13 pools per
Figure 6. Changes in flux rates and rate constants of plasma apoB lipoprotein metabolic pathways between hypertriglyceridemic patients and control subjects. Values represent apoB flux rates (A; mg d⁻¹ kg⁻¹ day⁻¹) and rate constants (B; pools per day) in hypertriglyceridemic patients. Numbers inside parentheses indicate percentage changes of these values vs control subjects. Dotted lines represent direct secretion into plasma of each lipoprotein type. Solid arrows connecting 2 plasma lipoprotein compartments represent lipolytic conversion from the originating compartment to the receiving compartment. Arrows exiting a compartment without going into another compartment represent direct removal pathways (ie, clearance from circulation). Numbers inside ovals in A indicate percentage changes in compartment pool sizes. *P<0.05, †P<0.01 between hypertriglyceridemic patients and control subjects by unpaired t test. LDL-1 indicates light LDL; LDL-2, medium LDL; LDL-3, dense LDL.
day; $P<0.05$; Figure 5). Because $>95\%$ of all LDL-3 are $E^{-CIII-}$, the FCR of total LDL-3 was also significantly lower in hypertriglyceridemic patients (0.72 ± 0.12 versus 1.11 ± 0.12 pools per day; $P<0.05$).

**Plasma Triglyceride Is Predicted by Altered Pattern of ApoB Lipoprotein Secretion**

We studied kinetic determinants of plasma triglyceride by linear regression. Secretion rates of apoE-containing TRL negatively predicted plasma triglyceride concentration, whereas secretion rate of TRL with apoC-III but not apoE positively predicted plasma triglyceride (Figure 7, top). These secretion rates still significantly predicted plasma triglyceride in multiple linear regression analysis after adjustment for body mass index and gender or plasma glucose, insulin, or HOMA index (data not shown).

**Plasma Triglyceride Is Dependent on ApoB Lipoprotein FCRs**

Linear regression analysis also showed that the FCR of E+CIII+ TRL (Figure 7, bottom left) or FCR of total VLDL negatively predicted plasma triglyceride, indicating that reduced VLDL FCR contributed to hypertriglyceridemia. In addition, because of the close association between hypertriglyceridemia and the small, dense LDL phenotype, we explored the relationship between plasma triglyceride and the kinetics of E+CIII- LDL-3, the smallest and predominant LDL type, and found that E+CIII- LDL-3 FCR negatively predicted plasma triglyceride (Figure 7, bottom right). Furthermore, in multiple linear regression analysis after adjustment for body mass index and gender or plasma glucose, insulin, or HOMA index, the FCRs of E+CIII+ TRL or E+CIII- LDL-3 still significantly predicted plasma triglyceride (data not shown).

**Enrichment of ApoCs but Not apoE in TRL in Hypertriglyceridemia**

ApoE-containing TRL had on average $\sim$10 to 30 molecules of apoE per particle, measured as molar ratio of apoE to apoB, similar in hypertriglyceridemic patients and normotriglyceridemic control subjects (Table 3). In contrast, hypertriglyceridemic patients had on average more than twice as many apoC-III molecules per particle as control subjects. TRLs were also enriched in apoC-I and apoC-II in hypertriglyceridemic patients.

**Discussion**

We explored apoB lipoprotein metabolism in a group of patients with moderate hypertriglyceridemia but normal plasma LDL cholesterol levels. Our results reveal major perturbations in both TRL and LDL metabolism that involve apoC-III and apoE. The defects in TRL metabolism in hypertriglyceridemia are 2-fold: (1) a shift of secretion pattern from apoE-containing TRL, lipoproteins...
that are quickly removed from the circulation before reaching LDL, to TRLs that do not contain apoE and are retained in the circulation longer while being converted to denser lipoproteins, and (2) reduced rate constants for clearance in apoE-containing TRL. These 2 metabolic defects result in reduced clearance and thus accumulation of TRLs, hence hypertriglyceridemia. In addition, hypertriglyceridemic patients also witness a shift in LDL distribution to dense particles, a phenotype that is caused by a quartet of factors: high rate of formation in plasma of LDL from apoC-III–containing triglyceride-rich VLDL via stepwise delipidation, shift in hepatic secretion from light to dense LDL, increased conversion in plasma of light to dense LDL, and slow clearance rate of dense LDL. We illustrated these metabolic defects in Figure 8.

Consistent with our earlier observations,13,15 this study demonstrates again that apoC-III and apoE are crucial regulators of apoB lipoprotein metabolism. Possession of apoE is key for direct clearance of TRLs. ApoE-containing TRLs mostly undergo direct removal and have a low tendency for LDL formation, whereas apoC-III antagonizes the action of apoE, channeling particles to lipolysis and LDL formation instead. TRLs with apoC-III but not apoE have minimal direct clearance and are metabolized quickly to LDL. TRLs without apoE or apoC-III are also nearly exclusively metabolized into LDL but with slower rates. This fundamental structure of apoB lipoprotein metabolism not only is at work under normal physiology13 but also explains metabolic changes during dietary intervention15 and in patients with hypertriglyceridemia.

Results of this study combined with our previous findings indicate that dietary carbohydrate could elicit changes in apoB lipoprotein metabolism in a manner parallel to endogenous hypertriglyceridemia. Previously, we have demonstrated that isocaloric substitution of dietary mono-unsaturated fat with carbohydrates significantly increases plasma levels of TRL with apoC-III but not apoE while reducing TRL with apoE.15 These phenotypic changes are qualitatively similar to what we observed in the present study in hypertriglyceridemic patients compared with normotriglyceridemic control subjects. Moreover, the dietary carbohydrate–induced TRL redistribution in plasma from apoE containing to those without apoE is caused by a corresponding shift in the liver secretion pattern, just as what the present study finds in hypertriglyceridemia compared with normotriglyceridemia. Therefore, hypertriglyceridemia is associated with changes in apoB lipoprotein metabolism that mimic the effects of dietary carbohydrates.

The observation that dietary intervention and hypertriglyceridemia induce similar phenotypic and mechanistic changes in apoB lipoprotein metabolism has clear physiological and interventional implications. For hypertriglyceridemic patients, consumption of a carbohydrate-rich diet seems to exacerbate an already unfavorable TRL secretion profile; on top of increased secretion of TRLs that have long circulation time and little direct removal (E−CIII− and E−CIII−), dietary carbohydrates also reduce the secretion of TRL that have short circulation time and high tendency for removal (E+CIII+ and E+CIII−), resulting in reduced clearance and increased accumulation of TRLs. Conversely, for hypertriglyceridemic patients, replacing dietary carbohydrates with monounsaturated fat may help remedy the unfavorable domination by anticlearance apoC-III to a more normal proclearance apoE-mediated apoB lipoprotein system. This is exactly what we have

### Table 3. Apolipoprotein Contents of TRL

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NTG indicates normotriglyceridemic control subjects; HTG, hypertriglyceridemic patients. Data represent the number of apolipoproteins per particle in TRL as calculated by molar ratios of corresponding apolipoprotein vs apoB based on plasma concentrations of these apolipoproteins and apoB. Data represent mean±SEM.

*P<0.05 by unpaired t test.
Figure 8. Major metabolic pathways for apoB lipoproteins in normotriglyceridemia (NTG) and their alteration in hypertriglyceridemia (HTG) leading to the formation of the dense LDL phenotype. Shown are the apoB lipoprotein metabolic pathways in NTG (A) and changes in HTG (B). Width of the arrows indicates the relative amount of flux through each pathway. The plus and minus signs indicate significant changes in flux in corresponding pathways in HTG vs NTG. Liver secretion of TRL (VLDL and IDL) shifts from apoE-containing TRL to TRL without apoE in hypertriglyceridemia, resulting in reduced TRL clearance and increased conversion of TRL to LDL. Accumulation of dense LDL is caused by a quartet of metabolic disturbances: increased flux from apoC-III-containing TRL to dense LDL, increased conversion of light and medium LDL to dense LDL, increased direct liver secretion of dense LDL E−CIII− and E−CIII+, and reduced clearance of dense LDL. E+CIII+ apoB lipoproteins make up only a minor fraction of plasma apoB lipoproteins and thus are not included.
observed: Hypertriglyceridemic patients eating a monoun-
saturated fat-rich diet do not have low secretion of apoE-
containing TRLs, in contrast with findings of the present
study, which has been carried out in subjects consuming a
high-carbohydrates diet.

In addition to macronutrients and hypertriglyceridemia,
other physiological or environmental conditions may also
affect TRL and LDL subfraction metabolism through similar
mechanisms that are closely regulated by apoC-III and apoE.
Ginsberg and colleagues elegantly studied the effects of weight loss on VLDL and LDL kinetics in a
group of hypertriglyceridemic patients. One major finding
is that normalization of plasma triglyceride by weight loss
is associated with reduced flux from VLDL to LDL and proportionately increased liver secretion of LDL, similar
to what we find in normotriglyceridemic compared with
hypertriglyceridemic subjects. In addition, we have previously studied the effects of estrogen treatment on apoB
lipoprotein metabolism in a group of healthy postmeno-
pausal women. It is interesting that oral estrogen, presumably having a totally different mode of action than
dietary carbohydrates or endogenous hypertriglyceridemia,
duces secretion of light VLDL and channels its flux through
dense VLDL to IDL to dense LDL, similar to what the present
study finds. In other words, it is possible that hyperproduction of
TRL, whatever the instigator, causes this flux alteration
to produce dense LDL. ApoC-III and apoE may play important
roles in these conditions.

This study sheds new light on the complex mechanisms
behind the formation of dense LDL. Hypertriglyceridemia
is usually associated with a shift of the LDL distribution
from lighter to denser particles, and this pattern of elevated
plasma triglyceride, a predominance of dense LDL, and
low high-density lipoprotein cholesterol is proposed as a
distinct form of dyslipidemia called the atherogenic li-
poprotein phenotype. Fisher et al., Packard and colleagues,
and Vega et al hypothesized from kinetic studies that dense LDL is formed from the substantial light
VLDL production, heightened activity of cholesteryl ester transfer protein-mediated exchange of lipids between light
VLDL and LDL, and finally the conversion of the triglyc-
eride-enriched, cholesteryl ester–depleted light LDL to
dense LDL via the function of hepatic lipase. They and
others demonstrated a major flux pathway from light
VLDL to dense LDL with little removal of intermediates,
whereas in contrast, normotriglyceridemic subjects have
much more direct LDL production and removal of dense
VLDL and LDL. This study suggests that apoC-III and
apoE are fundamental elements that produce this pathway.
In hypertriglyceridemia, we observe a high secretion rate
of apoC-III–containing light VLDL that does not have apoE.
The high apoC-III and low apoE together channel these light VLDL to smaller lipoproteins all the way to
dense LDL because apoC-III blocks clearance pathways
mediated by apoB and apoE. Consistent with these results,
Mauger and colleagues found that a high secretion rate of
apoC-III into plasma is associated with a preponderance of
dense LDL. The relative rarity of apoE in VLDL and IDL
of hypertriglyceridemic patients excludes a major clear-
ance mechanism. Further enhancing dense LDL formation
is increased conversion rate in plasma of light to dense
LDL presumably mediated by the typically high hepatic
lipase activity in patients with high triglyceride levels.
We have also found additional novel metabolic perturba-
tions that increase dense LDL in hypertriglyceridemia, ie,
a shift in hepatic LDL secretion from light to dense
particles, and selectively reduced clearance rate of the
predominant dense LDL.

Obesity and insulin resistance are potential confounders
of our results because the hypertriglyceridemic patients
had higher body mass index and HOMA index. However,
after these factors are controlled for, the strong relationships
between apoB kinetics parameters and plasma tri-
glyceride remain significant. Therefore, we believe that
our kinetics findings in the present study are relevant to
typical hypertriglyceridemic patients with obesity or insul-
lin resistance, and perhaps with type 2 diabetes.

One limitation of the present study is that our patients have
only moderately elevated fasting plasma triglyceride. They
have normal plasma cholesterol and apoB. However, normal
apoB concentrations are estimated to be present in two thirds
of hypertriglyceridemic patients, and participants of this
study represent this common subgroup of hypertriglyceride-
ia. This study does not include patients suffering mixed
hyperlipidemia, hyper-apoB, fatty liver, or familial hypertri-
glyceridemia. In these patients, additional mechanisms may
be involved to support hypertriglyceridemia. However, be-
cause elevated plasma and TRL apoC-III are common features
underlying various hypertriglyceridemic conditions, we suspect
that key findings of the present study, ie, a shift of TRL
metabolism from apoE to apoC-III and mechanisms for the
formation of the dense LDL phenotype, could also be at play.

Our detailed separation of plasma apoB lipoproteins into
24 subfractions led to the generation of many kinetic
parameters, ie, rate constants and flux rates. Special care is
necessary when reporting multiple outcomes because a
large number of unadjusted tests increases the susceptibil-
ity to type I error. However, tracer kinetic studies are
unique in that the main outcomes, ie, kinetics parameters,
are often interdependent. Computation of kinetic parame-
ters during multicompartmental modeling is constrained by
the metabolic connections between each lipoprotein com-
partment; thus, these parameters are dependent on each
other. Furthermore, comparisons of these outcomes are
preplanned, are previously published for other studies, and
are not opportunistic. These considerations mitigate the
risk of type I error.

Our study supports a central and causal role of apoC-III
in the pathogenesis of hypertriglyceridemia. Plasma apoC-
III levels in hypertriglyceridemic patients were on average
twice as high as in control subjects. ApoC-III transgenic
mice overproduce VLDL triglyceride. In humans, produc-
tion of VLDL apoC-III correlates strongly with secre-
tion of VLDL triglyceride. It has also been reported
recently that apoC-III can stimulate the secretion of
triglyceride-rich VLDL at the cellular level. In addition
to altered secretion, a main defect of TRL metabolism in
hypertriglyceridemia is the reduced catabolism of particles

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containing apoE. One likely mechanism could be the enrichment of apoC-III because we show that hypertriglyceridemic patients have on average twice as many apoC-III molecules on every apoE-containing VLDL. There is abundant evidence that apoC-III inhibits VLDL clearance. Taken together, these results indicate that apoC-III affects multiple major steps of triglyceride metabolism from secretion to clearance and may be an attractive pharmaceutical target to treat hypertriglyceridemia.

Finally, independently of its deleterious effects on apoB lipoprotein metabolism, our recent findings suggest that apoC-III exerts direct proatherogenic and proinflammatory effects on vascular cells, alone or as a component of apoB lipoproteins. Thus, the accumulation of plasma apoC-III and apoC-III-containing apoB lipoproteins in hypertriglyceridemic patients could expose them to greater risk for CHD. Therefore, elevated apoC-III may be a major mechanism linking hypertriglyceridemia to atherosclerosis and CHD.

Conclusions
ApoC-III and apoE play a central role in the pathogenesis of hypertriglyceridemia. In normotriglyceridemic participants, the majority of nascent TRL is secreted together with apoE and undergoes fast removal as a result of the strong proclearance functions of apoE. In contrast, in patients with moderate hypertriglyceridemia, TRL metabolism shifts from an apoE-dominated system to an apoC-III–dominated system as the majority of nascent lipoproteins are secreted without apoE but with apoC-III. ApoC-III antagonizes apoE- and apoB-induced lipoprotein clearance, an action that results in reduced clearance and consequently accumulation of atherogenic TRLs and their remnants that contain apoC-III. Formation of dense LDL, a feature of hypertriglyceridemia, is also linked to apoC-III via increased flux of the overproduced apoC-III–containing VLDL through lipolytic pathways to dense LDL. These adverse changes in apoB lipoprotein metabolism, in addition to the newly established proatherogenic effects of apoC-III itself, directly link hypertriglyceridemia to increased risk for CHD.

Acknowledgments
We thank Tae Kim, Janis Swain, and Helen Judge-Ellis for their technical assistance.

Sources of Funding
This work was supported by National Institutes of Health grants R01-HL-34980, R01-HL-56210, and RR02635.

Disclosures
Dr Sacks is a consultant to ISIS Pharmaceuticals and Genzyme and previously received a research grant from ISIS on apoC-III. The other authors report no conflicts.

References
7. Cohn JS, Patterson BW, Ueffelman KD, Davignon J, Steiner G. Rate of production of plasma and very-low-density lipoprotein (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male subjects with different body weights and levels of insulin sensitivity. J Clin Endocrinol Metab. 2004;89:3949–3955.


**CLINICAL PERSPECTIVE**

Hypertriglyceridemia is a common form of dyslipidemia that is frequently linked to premature coronary artery disease. Hypertriglyceremic patients often exhibit a small, dense low-density lipoprotein (LDL) phenotype. Our previous investigations demonstrate that apolipoprotein (apo) C-III and E, surface protein components of very low-density lipoprotein (VLDL) and LDL, play a dominant role in regulating the metabolism of these lipoproteins. In this work, we studied VLDL and LDL metabolism by the kinetics of its principal protein component, apoB, in 9 patients with moderate hypertriglyceridemia and 12 normotriglyceridemic control subjects using stable isotope labeling. Our results support a central role for apoC-III in VLDL and LDL metabolic defects leading to hypertriglyceridemia. Triglyceride-rich lipoprotein metabolism shifts from an apoE-dominated system in normolipidemic participants characterized by rapid clearance from the circulation of VLDL to an apoC-III-dominated system in hypertriglyceridemic patients characterized by reduced clearance of triglyceride-rich lipoproteins that are channeled to formation of dense LDL in plasma. In addition, apoC-III contributes to the formation of the dense LDL phenotype through a quartet of kinetic perturbations. These results indicate that the action of apoC-III to retard clearance of triglyceride-rich lipoproteins is a central metabolic feature underlying major changes in VLDL and LDL metabolism in hypertriglyceridemia. These adverse changes in apoB lipoprotein metabolism caused by apoC-III, in addition to the newly established proatherogenic effects of apoC-III itself, directly link moderate hypertriglyceridemia to increased risk for coronary heart disease. Therefore, modulating apoC-III may not only improve lipid profiles but also prevent the development of atherosclerotic plaques and their acute thrombotic complications.
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Chunyu Zheng, Christina Khoo, Jeremy Furtado and Frank M. Sacks

Circulation. 2010;121:1722-1734; originally published online April 5, 2010;
doi: 10.1161/CIRCULATIONAHA.109.875807

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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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SUPPLEMENT MATERIAL TO:

Apolipoprotein C-III and the metabolic basis for hypertriglyceridemia and the dense LDL phenotype

Chunyu Zheng, ScD; Christina Khoo, PhD; Jeremy Furtado, ScD; Frank M. Sacks, MD

From the Department of Nutrition, Harvard School of Public Health, Boston, MA (C.Z., C.K., J.F., F.M.S.); the Center for Excellence in Vascular Biology, Cardiovascular Division, Brigham and Women’s Hospital, Harvard Medical School, Boston MA (C.Z.). Current address for Dr. Khoo is Ocean Spray Inc., Lakeville-Middleboro, MA.
Supplemental Methods:

**Subjects:** Nine patients with moderate hypertriglyceridemia and twelve normolipidemic participants were recruited into the study. Participants with secondary hyperlipidemia, diabetes, (APO)E2/E2, E4/E4, E2/E4 genotypes, and use of medications that affect lipid metabolism were excluded from the study. The study was approved by the Human Subjects Committees at Harvard School of Public Health and Brigham and Women’s Hospital. All participants gave informed consent.

**Controlled dietary intake:** All participants entered a 3-week controlled dietary period before starting the tracer studies. The diet consisted of 20% energy from fat (7% from saturated fat, 8% from mono-unsaturated fat, and 5% from polyunsaturated fat), 65% from carbohydrate (48% from complex carbohydrate, and 17% from simple carbohydrate) and 15% from protein. The entire diet was provided to the participants as outpatients, and they were asked not to consume alcoholic beverages or any other source of caloric intake. Dietary energy levels were adjusted to reports of hunger or satiety, and trends in body weight which were measured every other day. The diet was prepared by the metabolic kitchen of the General Clinical Research Center at Brigham and Women’s Hospital (Boston, MA).

**Tracer infusion:** Participants were admitted to the General Clinical Research Center of Brigham and Women’s Hospital in the evening prior to study. After an overnight fast, they received a priming dose of 4.2 µmol/kg [D$_3$] L-Leucine (Tracer Technologies, Cambridge, MA), followed by a constant infusion of [D$_3$] L-Leucine leucine at 4.8 µmol/kg/hour for 15 hours. A bolus injection of [D$_5$] L-Phenylalanine, 7.3 µmol /kg, was also administered at the same time. We used two tracer techniques mainly
to reduce measurement errors. Both tracers, when modeled independently, gave similar parameter estimates in this study and previous ones \(^1,2\). But by combining both tracer data in the same model, the coefficients of variation for parameter estimates were substantially lowered.

Blood samples were collected at baseline, every 20 minutes in the first 2 hours after the infusion started, and hourly thereafter. For the first 4 hours, participants were restricted to noncaloric drinks. After this, they were given a standardized lunch and supper that had no fat, leucine or phenylalanine, and that contained 60% of daily required calories to avoid discomfort and abnormal metabolic effects of prolonged fasting.

**Sequential immuno-affinity chromatography:** Plasma was first incubated with an anti-apoE immunoaffinity column made from an affinity purified polyclonal antibody against human apoE (Academy-Biomedical, Houston TX). The unbound fraction (E-) and the bound fraction (E+) were then incubated with anti-apoC-III columns. This sequential immuno-affinity column procedure separated plasma into four immuno-fractions: those with both apoE and apoC-III (E+CIII+), those with apoE but not apoC-III (E+CIII-), those with apoC-III but not apoE (E-CIII+), and those without apoE or apoC-III (E-CIII-). Column efficiency was 98% for both apoE and apoC-III. Validation studies were performed to evaluate the effects of freezing and storage of plasma, and they were found not to affect the subsequent immuno-affinity columns \(^2,3\).

**Ultracentrifugation:** The above four immuno-fractions were then centrifuged separately at 25000 rpm on a Ti 25 rotor in an L8-70M instrument (Beckman, Brea CA) to isolate light VLDL (Svedberg units of flotation: 60–400), dense VLDL (S: 20–60), IDL (1.006–1.025 g/mL), LDL-1 (1.025–1.032 g/mL), LDL-2 (1.032–1.038 g/mL), and
LDL-3 (1.038–1.050 g/mL) using a modification of the Lindgren methods. A density of 1.050 g/mL was selected as the cutoff point for LDL to avoid contamination by Lp(a), which is concentrated at densities between 1.050–1.080 g/mL.

**Determination of lipids and apolipoproteins:** Triglyceride and cholesterol concentrations were determined enzymatically by Infinity™ kits (Thermo Electron, Melbourne Australia). Concentrations of apoE, apoC-III, and apoB were determined by sandwich ELISA using affinity-purified antibodies (Academy-Biomedical, Houston TX). Intra-assay coefficients of variances for lipid and apolipoprotein measurements were less than 5%, and inter-assay coefficients of variances were less than 10%.

Plasma glucose was determined enzymatically by a Glucose Assay kit (Sigma, St. Louis, MO). Insulin was determined by an ELISA kit from Abcam (Cambridge, MA).

**Measurement of tracer enrichment and pool size:** Apolipoprotein B was precipitated from the lipoprotein fractions, and tracer enrichment and tracee mass were measured by GC-MS as described previously. In all participants, plasma apoB concentrations stayed constant during the infusion protocol with less than 10% variation. Under the study dietary conditions, there is a negligible contribution of apoB48 to the precipitated apoB in the VLDL (<5%) and LDL (<1%) fractions. For participants with body mass index (BMI) between 18.5–29.9 kg·m⁻², plasma volume (L) was assumed to be 4.4% of body weight (kg). For obese participants (BMI>30.0), plasma volume was calculated by an equation described by Nikkila and Kekki: plasma volume (L) = ideal body weight (kg) X 0.044 + excess weight (kg) X 0.010.

**Model development and kinetic analysis:** Tracer enrichment and apoB were measured in 24 apoB lipoprotein fractions (four immuno-fractions further separated into
six density fractions) for each sample. A multi-compartment model was employed to find
the best fit to the observed data by using SAAM II software, Version 1.2.1 (SAAM
Institute, Seattle WA) following established methods as described previously.

**Figure 2** shows the diagram of the model. Plasma apoB lipoproteins were represented by
21 compartments separated in density by ultracentrifugation and in apoE and apoC-III
content by immuno-affinity chromatography. Three LDL types, LDL-1, -2 and -3
containing apoE and not apoC-III, had concentrations that were too low to measure and
they were not studied.

The model was developed by designating every physically separated lipoprotein
fraction to a single compartment. A plasma amino acid forcing function was followed by
a hepatic intracellular delay compartment accounting for the time required for the
synthesis and secretion of apoB100 into plasma. The model allowed apoB secretion from
the liver into each plasma compartment, as well as direct clearance from the circulation.
Metabolism of plasma apoB lipoproteins includes conversion of larger particles to
smaller ones. Modeling results suggested that for apoB lipoproteins with the same apoE
and apoC-III composition, stepwise delipidation as the primary metabolic pathway
usually fitted the data best (e.g. E-CIII+ VLDL → E-CIII+ IDL → E-CIII+ LDL). In
addition, we also tested conversion pathways among lipoproteins with different apoE and
apoC-III compositions, and found some that were required to improve the fitting. For
example, conversion from E-CIII+ light VLDL to E-CIII- dense VLDL represents such a
pathway, indicating during lipolysis, some E-CIII+ light VLDL lose all of their apoC-III
content and are converted to dense VLDL without apoC-III. When evaluating these
potential conversion pathways between apoB lipoproteins with different apoE and apoC-
III composition, we first compared the enrichment curves of the originating and
destination compartments to eliminate pathways that prohibit a precursor-product
relationship. Next, qualified pathways were added to the existing model one by one, and
were fitted to the data. Pathways for which rate constants were zero or negligible were
eliminated. Average particle size and triglyceride content were also taken into
consideration when evaluating such a pathway.

After the model structure was established by the mean tracer and tracee data of all
participants, each participant’s data were fitted individually to obtain the parameter
values. For each individual, both $[D_5]$ L-Phenylalanine and $[D_3]$ L-Leucine tracer data
were included in the same model, and the data were solved simultaneously by making the
rate constants equal for leucine and phenylalanine experiments. Thus for each participant
a single set of rate constants was produced. This model was able to generate excellent fits
to tracer enrichments, and apoB masses. The coefficients of variation for most parameter
estimates were less than 25%, and for the major pathways usually less than 15%.

Regression analysis: To determine the major kinetics parameters that predict
plasma triglyceride levels, we performed simple and multiple linear regression analysis
with SAS (Version 9.1, SAS Institute, Cary NC). We used plasma triglyceride
concentration (mg/dL) as the dependent variable and apoB secretion rates or fractional
catabolic rates as independent variables. In multiple regression analysis, in addition to the
above kinetic parameters, independent variables also included body mass index and
gender, or fasting glucose, fasting insulin, or HOMA index.

Statistical analysis: Comparisons between hypertriglyceridemics and controls
were performed using unpaired t-test with SAS software (Version 8). The results are
presented as mean ± standard error of the mean (SEM) unless specified otherwise. A $p$-value $\leq 0.05$ (two-sided) is considered statistically significant.
Supplemental References:


**Supplemental Table 1: Comparison of kinetics parameters between hypertriglyceridemic patients and normotriglyceridemic controls.**

<table>
<thead>
<tr>
<th>Direct Removal Pathways</th>
<th>Flux Rates (mg·day⁻¹·kg⁻¹)</th>
<th>Rate Constants (pools·day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NTG Mean SEM</td>
<td>HTG Mean SEM</td>
</tr>
<tr>
<td>E-CIII- Va</td>
<td>0.06 0.04 0.28 0.18</td>
<td>0.166</td>
</tr>
<tr>
<td>E-CIII- Vb</td>
<td>0.35 0.08 0.53 0.15</td>
<td>0.327 0.15 0.42 0.25</td>
</tr>
<tr>
<td>E-CIII- IDL</td>
<td>0.21 0.10 1.30 0.20</td>
<td>0.007 0.11 1.14 0.47</td>
</tr>
<tr>
<td>E-CIII- LDL1</td>
<td>0.61 0.22 1.54 0.30</td>
<td>0.042 0.11 0.99 0.12</td>
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<tr>
<td>E-CIII- LDL2</td>
<td>2.12 0.50 1.53 0.34</td>
<td>-28% 0.446 0.42 0.78 0.22</td>
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<tr>
<td>E-CIII- LDL3</td>
<td>10.20 0.90 9.82 1.35</td>
<td>-4% 0.823 1.17 0.75 0.13</td>
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<tr>
<td>E-CIII+ Va</td>
<td>0.02 0.00 0.05 0.09</td>
<td>0.278 0.25 0.17 0.21</td>
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<tr>
<td>E-CIII+ Vb</td>
<td>0.05 0.02 0.16 0.10</td>
<td>0.304 0.40 0.21 0.48</td>
</tr>
<tr>
<td>E-CIII+ IDL</td>
<td>0.10 0.05 0.33 0.15</td>
<td>0.179 0.38 0.20 0.64</td>
</tr>
<tr>
<td>E-CIII+ LDL1</td>
<td>0.20 0.08 0.35 0.20</td>
<td>0.523 0.58 0.19 0.75</td>
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<tr>
<td>E-CIII+ LDL2</td>
<td>0.13 0.06 0.12 0.05</td>
<td>-5% 0.950 0.33 0.20 0.39</td>
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<tr>
<td>E-CIII+ LDL3</td>
<td>0.13 0.06 0.27 0.11</td>
<td>103% 0.328 0.32 0.21 0.60</td>
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<tr>
<td>E+III- Va</td>
<td>0.40 0.07 0.25 0.06</td>
<td>-37% 0.205 9.36 1.72 2.77</td>
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<tr>
<td>E+III- Vb</td>
<td>0.65 0.14 0.31 0.08</td>
<td>-52% 0.109 0.19 0.21 0.60</td>
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<tr>
<td>E+III- IDL</td>
<td>0.39 0.05 0.25 0.08</td>
<td>-36% 0.179 0.77 1.29 5.23</td>
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<tr>
<td>E+III+ Va</td>
<td>3.26 0.51 1.58 0.24</td>
<td>-51% 0.037 0.820 0.75 3.68</td>
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<tr>
<td>E+III+ Vb</td>
<td>3.19 0.25 2.47 0.43</td>
<td>-22% 0.215 0.95 1.52 5.38</td>
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<tr>
<td>E+III+ IDL</td>
<td>2.39 0.23 1.44 0.27</td>
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<tr>
<td>E+III+ LDL1</td>
<td>1.22 0.18 0.48 0.07</td>
<td>-60% 0.011 0.37 0.01 0.35</td>
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<tr>
<td>E+III+ LDL2</td>
<td>0.35 0.06 0.30 0.06</td>
<td>-14% 0.643 2.34 0.50 5.07</td>
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<tr>
<td>E+III+ LDL3</td>
<td>0.24 0.03 0.20 0.05</td>
<td>-19% 0.477 1.96 0.56 3.33</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Conversion Pathways</th>
<th>Flux Rates (mg·day⁻¹·kg⁻¹)</th>
<th>Rate Constants (pools·day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NTG Mean SEM</td>
<td>HTG Mean SEM</td>
</tr>
<tr>
<td>E-CIII- Va to E-CIII- Vb</td>
<td>0.56 0.12 1.07 0.26</td>
<td>0.116 0.16 1.47 0.07</td>
</tr>
<tr>
<td>E-CIII+ Va to E-CIII- Vb</td>
<td>0.83 0.15 1.96 0.30</td>
<td>0.007 0.17 3.08 0.42</td>
</tr>
<tr>
<td>E-CIII- Vb to E-CIII- IDL</td>
<td>1.98 0.26 3.60 0.50</td>
<td>0.043 0.36 7.91 0.85</td>
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<tr>
<td>E-CIII+ Vb to E-CIII- IDL</td>
<td>2.15 0.28 2.69 0.37</td>
<td>0.331 0.83 1.83 5.86</td>
</tr>
<tr>
<td>E-CIII- LDL to E-CIII- LDL1</td>
<td>3.86 0.41 4.46 0.67</td>
<td>0.054 0.30 3.24 0.38</td>
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<tr>
<td>E-CIII+ LDL to E-CIII- LDL1</td>
<td>1.81 0.31 2.30 0.33</td>
<td>0.370 0.89 1.77 3.66</td>
</tr>
<tr>
<td>E+III+ IDL to E-CIII- LDL1</td>
<td>0.75 0.20 0.42 0.18</td>
<td>0.339 0.14 1.66 0.95</td>
</tr>
<tr>
<td>E-CIII- IDL to E-CIII- LDL2</td>
<td>1.63 0.49 1.26 0.41</td>
<td>-22% 0.656 1.56 0.52 1.72</td>
</tr>
<tr>
<td></td>
<td>ApoB flux rates (mg•day$^{-1}$•kg$^{-1}$) and rate constants (pools•day$^{-1}$) in hypertriglyceridemic patients (HTG, N=9) and normotriglyceridemic controls (NTG, N=12). “Change” column indicates percentage changes of HTG values compared to corresponding controls, i.e. (HTG-NTG)/NTG. P-values indicate difference between the two groups by unpaired t-test. *: $p&lt;0.05$, †: $p&lt;0.01$. Flux rates data were used to prepare Figure 6A, and rate constants data were used to prepare Figure 6B. Va: Light VLDL; Vb: Dense VLDL.</td>
<td></td>
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