Triglyceride-Rich Lipoprotein-Associated Apolipoprotein C-III Production Is Stimulated by Plasma Free Fatty Acids in Humans

Mirjana Pavlic, René Valéro, Hélène Duez, Changting Xiao, Linda Szeto, Bruce W. Patterson, Gary F. Lewis

Objective—Insulin resistant states are associated with increased fatty acid flux to liver and intestine, which stimulates the production of triglyceride-rich lipoproteins (TRL). ApoC-III production and plasma and TRL concentrations are increased in insulin resistance and may contribute to the hypertriglyceridemia of these conditions. The mechanism underlying that increase is not known, but because apoC-III and VLDL production are closely linked we hypothesized that FFAs may stimulate TRL apoC-III production.

Methods and Results—We used Intralipid/heparin (IH) to raise plasma FFA in 12 healthy men in the fed state, and stable isotopes to examine apoC-III metabolism. TRL apoC-III concentration was significantly higher in the IH study, and this increase was associated with higher production (PR) and fractional catabolic rate (FCR). The increase in production was greater than in FCR (90% versus 30%, respectively), accounting for the elevated concentration. Glycerol infusion had no effect on apoC-III concentration, PR, or FCR compared to saline, indicating that the effect was not attributable to glycerol released from intralipid.

Conclusion—These findings confirm that TRL apoC-III production is stimulated by an acute elevation of plasma FFAs, suggesting a novel regulatory pathway that may play a role in the overproduction of TRL apoC-III in insulin resistant states. (Arterioscler Thromb Vase Biol. 2008;28:1660-1665)

Key Words: apolipoprotein C3 ■ free fatty acids ■ insulin resistance ■ lipoprotein

Hypertriglyceridemia is a well described feature of insulin resistance and type 2 diabetes and is commonly associated with other lipid abnormalities such as low plasma HDL concentration and a shift toward smaller and denser LDL particles, as well as an increased risk of atherosclerotic cardiovascular disease.1 This hypertriglyceridemia is attributable predominantly to an increased rate of production of triglyceride-rich lipoproteins (TRL) by both the liver (reviewed in2) and intestine.3,4 Several factors contribute to the overproduction of TRL particles in these conditions; including increased flux of fatty acids to liver and intestine, increased de novo lipogenesis, increased stability of apoB, and overexpression of microsomal transfer protein (MTP), the latter contributing to the increased efficiency of lipoprotein assembly and secretion.5,6

Reduced clearance of the triglyceride moiety of TRL and of the particles themselves also contributes to the hypertriglyceridemia (reviewed in3), particularly when the metabolic control of type 2 diabetes is poor and in the postprandial state, when there is a large influx of intestinally-derived TRL particles. Functional deficiency of lipoprotein lipase (LPL) or competition for a common, saturable, lipolytic pathway has been shown to play an important contributing role in this impaired TRL clearance.7 Additionally, elevated apoC-III in insulin resistance and in type 2 diabetes8–14 has also been postulated to contribute to the impairment of TRL particle clearance, possibly by inhibiting the activity of LPL15 or by interfering with the interaction between TRL particles and hepatic lipoprotein receptors.16,17 Major insight into the function of apoC-III has been obtained from genetically modified mouse models, with overexpression of apoC-III resulting in severe hypertriglyceridemia18 and apoC-III deficiency resulting in hypotriglyceridemia.19

ApoC-III is synthesized mainly by the liver and to a lesser extent by the intestine (reviewed in20,21). Mature apoC-III is a 79 amino-acid glycoprotein present in VLDL, chylomicrons, and HDL, and has the ability to exchange between TG-rich lipoproteins and HDL.22 In normolipidemic subjects the majority of plasma apoC-III is bound to HDL whereas in hypertriglyceridemic subjects the majority is bound to TRL.21
Although apoC-III is a relatively minor component of HDL protein, approximately 50% of total plasma apoC-III may be in the HDL fraction.21 In humans, the APOA1/C3/A4/A5 gene cluster is located on chromosome 11q23.23 ApoC-III gene expression is normally downregulated by insulin24 via Foxo1.25 A variant promoter that is defective in its response to insulin results in overexpression of the apoC-III gene, contributing to the development of hypertriglyceridemia.26 These observations have led some to hypothesize that overexpression of apoC-III in insulin resistant states leads to an elevation of TRL apoC-III concentration and that this phenomenon is an important contributor to the highly prevalent hypertriglyceridemia that occurs in insulin resistance.26 In support of this notion, insulin sensitizing therapy with the PPARγ agonist pioglitazone in patients with type 2 diabetes reduced apoC-III production, plasma apoC-III concentration, and lowered plasma triglycerides,27 although it should be noted that pioglitazone also has some PPARα agonist activity and the drug may have mediated its effect on apoC-III production and triglyceride metabolism by a mechanism that is not dependent on insulin sensitization. Insulin resistance and apoC-III production are correlated in humans,11 and increased VLDL apoC-III concentration resulting from its overproduction is strongly associated with the delayed catabolism of triglycerides and apoB in VLDL.28

Fatty acid flux from adipose tissue is increased in insulin resistant states and plays an important role in stimulating the production of TRL particles by the liver.29,30 More recently, we have made the observation that acute elevation of plasma FFAs by Intralipid/heparin increased intestinal lipoprotein production in the Syrian Golden hamster30 and in humans.31 Because increased apoC-III production is closely linked with VLDL-triglyceride production,32 we hypothesized that TRL apoC-III production may also be stimulated by elevated plasma FFAs, providing an alternative mechanism explaining the increased rate of production of TRL apoC-III in insulin resistance. In the present study we examined the effect of an acute elevation of plasma FFAs on TRL apoC-III production in healthy men and found that TRL apoC-III production rate is indeed increased by an acute elevation of plasma FFAs.

Materials and Methods

Subjects

The subjects participating in the present study and the study protocol have been reported previously.31 The previous report of these subjects focused exclusively on the kinetics of TRL-apoB100 and TRL-apoB48 in response to an acute elevation of plasma FFA.31 The demographic characteristics and fasting biochemical profiles of the 12 study participants were described in detail in that publication,31 and only mean values are presented in Table 1 of the present article. All were healthy male subjects with no previous history of cardiovascular, gastrointestinal, or systemic illness, or surgical intervention within 6 months before the studies. No subject was taking medications, and they were all normoglycemic. To exclude those with gross dyslipidemia from the study, individuals whose total cholesterol was >5.3 mmol/L, TG >3 mmol/L, HDL-c <0.9 mmol/L, LDL-c >3.2 mmol/L, were excluded.

The Research Ethics Board of the University Health Network, University of Toronto, approved the study and all subjects gave written informed consent prior to their participation.

### Table 1. Demographic Characteristics and Mean Fasting Biochemical Profiles of Study Participants

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Weight, kg</th>
<th>Waist, cm</th>
<th>BMI, kg/m²</th>
<th>Glucose, mmol/l</th>
<th>Insulin, pmol/l</th>
<th>FFA, mmol/L</th>
<th>Plasma TC, mmol/L</th>
<th>Plasma TG, mmol/L</th>
<th>Plasma LDL-c, mmol/L</th>
<th>TRL apoC-III, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>43.1±4.9</td>
<td>76.4±8.4</td>
<td>98.0±2.8</td>
<td>24.5±2.7</td>
<td>4.5±0.48</td>
<td>52.1±7.4</td>
<td>0.5±0.01</td>
<td>4.08±0.45</td>
<td>1.24±0.21</td>
<td>2.59±0.16</td>
<td>4.72±0.84</td>
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<tr>
<td>30–57</td>
<td>66.8–101</td>
<td>87–112</td>
<td>22.6–31.7</td>
<td>4.3–5.5</td>
<td>27–89.5</td>
<td>0.2–1.0</td>
<td>4.0–5.25</td>
<td>0.72–2.87</td>
<td>1.6–3.17</td>
<td>1.57–14.7</td>
</tr>
</tbody>
</table>

### Experimental Protocol for Lipoprotein Kinetic Studies

The experimental protocol has previously been described in detail.31 Briefly, 12 subjects underwent 2 separate 12-hour lipoprotein turnover studies, both conducted during a constant fed state, in random order, 4 to 6 weeks apart. One study was performed during intravenous infusion of Intralipid (a synthetic triglyceride emulsion, suitable for intravenous infusion, that provides a source of mainly polyunsaturated FFAs) and heparin (to activate lipoprotein lipase, which stimulates intravascular lipolysis of the intralipid triglycerides), and the other during intravenous saline infusion as a control study. Because the infusion of Intralipid raises both FFAs and glycerol, a subset of 5 subjects also underwent a third kinetic study during i.v. infusion of glycerol as an additional control study to differentiate the effects of FFA from potential effects of glycerol released by intravenous lipolysis of Intralipid. In this case, all 3 studies were performed in random order, 4 to 6 weeks apart.

After an overnight fast, an intravenous catheter was inserted into a superficial vein in each forearm, one for infusion and one for blood sampling. At 4 AM the subject was instructed to start ingesting the first aliquot of a liquid food supplement, hourly for the first 3 hours (Hormel Great Shake Plus, Hormel Health Labs, 49% calories from carbohydrates, 13% from proteins), each hourly aliquot equivalent to 1/17th of their total daily caloric needs. After the first 3 hours (after 7 AM), the subjects ingested the same formula every half hour for the remainder of the study, ie, 1/34th of their daily caloric intake every half hour. Total energy requirements to achieve a constant fed state were determined using the Harris Benedict Equation. At 4 AM, at the same time as the start of the hourly formula ingestion, an i.v. infusion with either Intralipid/heparin (IH; Baxter; Intralipid 20% at 20 mL/h plus heparin 250U/h) or saline (65 mL/h) or glycerol (2.25 g/h) was started and was constantly infused for the full 17-hour duration of the study. Five hours after starting the liquid formula ingestion (at 9 AM), a primed constant infusion of dextrose-labeled leucine33 (1D3L-leucine 98%, Cambridge Isotope Laboratories) was administered (10 μmol/kg bolus followed by 10 μmol/kg.hr for 12 hours) to enrich apoC-III to calculate production and clearance rates, as previously described.34 Blood samples were collected at regular intervals as previously described.31 The total blood volume drawn during the entire study was <350 mL. The hematocrit does not change appreciably after removal of this amount of blood.

For details on laboratory methods, calculation of lipoprotein clearance and production rates by multicompartmental modeling, and statistical analysis, please see the data supplement (available online at http://atvb.ahajournals.org).

### Results

Glycerol Control Study

We have recently demonstrated that glycerol infusion has no effect on plasma FFA, TG, TRL-apoB100, or TRL apoB48...
levels, production and clearance rates compared to saline.31 Here we found no difference between glycerol and saline infusion on TRL apoC-III concentration (saline 5.52 ± 0.9 versus glycerol 6.0 ± 0.46 mg/dL, P = 0.464), production (saline 2.54 ± 0.63 versus glycerol 2.14 ± 0.20 mg/kg · d, P = 0.617), and clearance (saline 0.99 ± 0.11 versus glycerol 0.80 ± 0.06 pool/d, P = 0.300). For clarity, and because power calculation estimated n = 64 subjects would be needed to detect a significant difference between the kinetics parameters for TRL apoC-III in the saline and glycerol studies at P = 0.05 with 80% power, the following results and illustration will therefore compare only the Intralipid/heparin and saline studies.

### Discussion

There is a positive association between TRL apoC-III and TRL triglyceride concentrations and production rates,21,31 and also TRL apo-B concentrations and production rates,28,32,35
which are increased in insulin resistant states. Elevated FFA flux from extrahepatic tissues is one important cause of the overproduction of VLDL in insulin resistance,6,29,31,36,37 and we have recently shown in the Syrian Golden hamster30 and in humans31 that intestinal lipoprotein production is also stimulated by an acute elevation of plasma FFAs. Here, we examined whether an acute elevation of FFAs also stimulates TRL apoC-III production in healthy humans. Our findings confirm that TRL-associated apoC-III production is stimulated by an acute elevation of plasma FFAs, suggesting a novel regulatory pathway that may play a role in the overproduction of TRL apoC-III in insulin resistant states.

Kinetics studies in human subjects have shown that increased production rather than decreased clearance of apoC-III is the most important determinant of the elevated plasma apoC-III levels that characterize patients with hypertriglyceridemia and features of insulin resistance.11,32 We used Intralipid/heparin infusion to raise plasma FFA concentration in the fed state and well established stable isotope enrichment techniques to examine TRL apoC-III metabolism. The TRL apoC-III concentration was significantly higher in the IH infusion study compared with saline control study, and this increase was associated with both higher production rates of TRL apoC-III as well as higher FCR, with the increase in production rate being of greater relative magnitude (90% higher FCR in IH versus saline), providing an explanation for the increase in concentrations. Glycerol had no effect on apoC-III concentration, production or clearance rates when compared to saline infusion, indicating that the effect of IH was attributable to the FFAs released from the synthetic TG emulsion by the action of heparin-stimulated LPL, and not attributable to the release of glycerol.

Unlike apoB, apoC-III and other apolipoproteins are able to exchange actively between lipid surfaces, and apoC-III redistribution to intralipid particles must be considered in the interpretation of our findings. Larger intralipid particles would be expected to contain apoC-III and other exchangeable apolipoproteins, as has been demonstrated by others in the past.38,39 Whereas newly secreted apoC-III is very likely to be rapidly transferred to intralipid particles, there is no known mechanism for apolipoprotein feeding back on its own gene transcription, and therefore by far the most likely culprit responsible for enhancing apoC-III production by 90% in our studies is still the FFA released by intravascular lipolysis of intralipid. As shown in Figure 1, there was a slight decline in HDL apoC-III during the Intralipid/heparin infusion experiment. This decline likely reflects the transfer of HDL apoC-III to the increased pool of TRL and intralipid particles. Because the residence time of HDL apoC-III has been shown to be 3 times longer than VLDL apoC-III,34 and if intralipid particle-associated apoC-III is also cleared faster than HDL apoC-III because of enhanced lipolysis of intralipid by heparin-stimulated LPL, this transfer of apoC-III from HDL to TRL could account for the 30% higher FCR that we documented during Intralipid/heparin versus saline infusion.

Limitations of the present study must be addressed. First, for practical reasons the study was designed to examine the effect of a short-term elevation of plasma FFAs on lipoprotein metabolism. It is not feasible nor is it technically possible, considering issues of stable isotope recycling that complicate interpretation of data with prolonged infusion, to examine longer term elevations of plasma FFAs on lipoprotein-associated apolipoprotein kinetics. Extrapolation of our findings to clinical conditions that are associated with chronic elevations of plasma FFAs, such as insulin resistance and type 2 diabetes, should therefore be made with appropriate caution. The levels of FFA achieved in the present study were well within the physiological range that occurs in those with insulin resistance or type 2 diabetes. Second, our study was conducted in the fed state to facilitate the assessment of TRL apoB48 kinetics (reported in32). This was necessary because of the technical challenge of measuring very low concentrations of apoB-48-containing particles. Our finding that TRL apoC-III production rates were increased in the IH study and stimulated by acute elevation of plasma FFA were made in the setting of high-fat feeding and ongoing fat absorption. We cannot say with certainty that the same would have been observed in the fasted state. This would have to be specifically examined in future studies. Third, because apoC-III is produced in both liver and intestine, we were unable to determine whether the increase in production occurred primarily in the liver, primarily in the intestine, or in both organs to a relatively similar extent. Fourth, an in vivo human study of this nature cannot examine the molecular mechanisms of the increased apoC-III production and cannot determine whether the increase occurred as a result of upregulation of apoC-III gene transcription or whether the effect occurred at the posttranslational level. In addition we cannot determine whether the increase in production of TRL apoC-III occurred.

Figure 2. Effect of Intralipid/heparin infusion on mean TRL apoC-III fractional catabolic (FCR) and production rates (PR). TRL apoC-III FCR (A) and PR (B) were determined at steady state in subjects receiving either Intralipid/heparin (black bars) or saline infusion (white bars). Both TRL apoC-III FCR and PR were significantly increased by the Intralipid/heparin infusion compared to saline (*P=0.001 and *P=0.003, respectively).
as a direct result of fatty acids stimulating the hepatocyte or intestinal enterocyte, was mediated through one or a number of intermediary regulators of apoC-III secretion, or whether the effect occurred secondary to the well described insulin resistance that occurs with elevation of plasma FFAs. Given our findings of a simultaneous upregulation of TRL apoB48, apoB100, and apoC-III production in response to the elevation of plasma FFAs, and because the regulation of apoB48 and apoB100 by fatty acids has been shown to occur primarily at the posttranslational level by inhibition of apoB degradation and enhancement of lipoprotein assembly, it is quite possible that the increase in apoC-III production seen in the present study also occurred posttranslationally. This interesting issue remains to be determined in future in vitro or ex vivo mechanistic studies.

As can be seen in Figure 1, TRL apoC-III concentration (pool size, on which our kinetic modeling was based) was not in perfect steady state during the 12-hour kinetics study (5 to 17 hours). We turned to model simulations to assess the potential impact of a nonsteady state on the analysis of apoC-III turnover kinetics, as we have described and illustrated in the online Data Supplement. Based on these model simulations and for the reasons described in the online Data Supplement, we are confident that our calculated production rates are valid and are not affected by the small perturbations of steady state that occurred in this study.

In conclusion, the present report provides evidence that TRL apo-CIII production is stimulated by a short-term elevation of plasma FFAs in healthy men. It is reasonable to assume that chronic elevation of plasma FFAs seen in type 2 diabetes and insulin resistance could play an important role in TRL apoC-III overproduction, which in turn could aggravate the hypertriglyceridemia of insulin resistant states and type 2 diabetes. Given the complexity of the mechanisms that have been shown to contribute to the overproduction of intestinal and hepatic lipoproteins in these conditions, it is unlikely that TRL apoC-III overproduction is the sole or even the dominant mechanism accounting for the hypertriglyceridemia, but it certainly could contribute and should be considered a potential therapeutic target for ameliorating this atherogenic condition.

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Disclosures
None.

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Laboratory methods

Plasma was separated from blood within 2 hours in a refrigerated centrifuge at 2000 rpm for 15 min at 4°C. TRL were isolated at each time point at d=1.006 for 16 hrs, 39,000 rpm at 12°C. Plasma amino acids were recovered from 0.25ml plasma after precipitation of plasma proteins with 1.8ml acetone and extraction of the aqueous phase with hexane. The aqueous phase was dried under vacuum and amino acids derivatised and enrichments determined as described below. Proteins in the TRL fraction were delipidated using methanol/diethyl ether. ApoC-III was isolated from TRL by preparative isoelectric focusing (IEF) on polyacrylamide-urea gels (pH gradient 4-7), stained with Coomasie blue stain and destained with 10% acetic acid. The band corresponding to the major isoform of apoC-III, apoC-III₁, was excised and added to a borosilicate sample vial for further processing (representative gels from the IH and saline studies with clear separation of the apoC-III bands are shown in Fig S1B.)

Gels were hydrolysed with 6N HCl for 24h at 110°C, cooled to -20°C for 20 min and centrifuged at 3500rpm for 10 min. Samples were dried under vacuum and then derivatized by adding 100µl 1:1 acetonitrile (ACN):MTBSTFA (N-tert-butylidimethyl-N-methyltrifluoracetamide; Sigma-Aldrich) mixture and incubating 30 min at 70°C. tBDMS derivatives were determined by electron impact ionization GC/MS (Agilent 5975/6890N GC/MS, Agilent Technologies Canada INC, Mississauga, Ontario, Canada). Chromatographic separations were preformed on a 30mx0.25mm Agilent DB-17MS column with the helium carrier
gas flow 1ml/min, and the temperature programmed, following an initial hold at 80°C for 1 min to raise from 80°C to 150°C at 70°C/min then to 250°C at 20°C/min and then ramped to 300°C at 70°C/min. Selective ion monitoring at m/z = 200 and 203 (ionic species corresponding to derivatized non deuterium-labeled and deuterium-labeled leucine respectively) was performed, and the enrichment data were converted to tracer-to-tracee ratios by measurement of appropriate isotopic enrichment standards. To minimize artifacts, calibration with standards of known isotopic enrichments was performed with each set of samples and a blank gel was run to ensure leucine peak is always <5% (blank TTR mean ±SEM 2.32±0.07%).

Insulin, glucose and lipid concentrations in plasma, TRL and HDL were determined as we have previously described. Total and TRL-apoC-III concentrations were determined by an automated (MODULAR P automaton-Roche-France) immunoturbidimetric assay. Antihuman apoC-III polyclonal antibodies from goat were used according to the manufacturer’s recommendations (K-Assay, Kamiya biomedical company, USA).

**Calculation of lipoprotein clearance and production rates by multicompartmental modeling**

Stable isotope enrichment time course curves for apoC-III were fitted to a three compartment model using SAAM II computer software (version 1.2; University of Washington, Seattle, WA). Compartment 1 represents the plasma amino acid precursor pool. Compartment 2 is an intracellular delay compartment, which accounts for apoC-III synthesis lipoprotein assembly and secretion.
Compartment 3 represents circulating plasma lipoproteins (Figure S1 A). We assumed constant enrichment of the precursor pool and also that TRL apoC-III attained similar enrichment plateau as TRL apoB48, which we previously estimated to be approximately 60% of the plasma amino acid enrichment \(^4,6\). Therefore, the peak enrichment attainable by TRL apoC-III in this study was set to 60% of the plasma amino acid enrichment. This approach is not likely to affect the overall conclusions of the study regarding the effect of plasma FFA on apoC-III PR. Each subject was in steady state with respect to TRL apoC-III concentrations during the 12-hr kinetic study, so fractional catabolic rate (FCR) was equivalent to fractional synthetic rate. Kinetics parameters were derived from individual enrichment time course curves (Figure S1 C). Production rates were calculated using the FCR of TRL apoC-III multiplied by pool size measured over the 12 hours of the study where pool size = average plasma concentration (mg/dl) between t5hr and t17h of the experimental protocol x plasma volume/kg body weight (plasma volume is estimated as 0.045 liter/kg).

As can be seen in figure 1 of the manuscript, TRL apoC-III concentration (pool size, upon which our kinetic modeling was based) was not in perfect steady state during the 12hr kinetics study (5 to 17hrs). We turned to model simulations to assess the potential impact of a non-steady state on the analysis of apoC-III turnover kinetics. The model assumed a homogeneous single pool for apoC-III, and production from a source of labeled leucine that was held to a constant TTR=2%. In the first solution we simulated a metabolic steady state, with constant absolute production and clearance rates and a fractional clearance rate.
of 0.5 h\(^{-1}\). In the second solution, the absolute clearance rate was kept constant and the absolute production rate increased at a linear rate over the time course, resulting in a final pool size of apoC-III after 10 hours that was triple the starting value. In the third solution, the absolute production rate was kept constant and the absolute clearance rate decreased in a linear rate over the time course, such that the final pool size again was 3 times the initial pool size (note that the fractional clearance rates changed non-linearly in the latter two solutions). Both non-steady state solutions show a rise toward the same TTR plateau (see data supplement for figure S2). The time course for change in pool size in the two non-steady state solutions was identical; thus, it would not be possible to distinguish between these non-steady state solutions even if time-dependent values for apoC-III concentration were included in the model. Note that if perfect, noiseless TTR values could be obtained, it is theoretically possible to distinguish between these 3 solutions (theoretical identifiability). Given the noise that is inherent in measuring apoC-III TTR, however, it would not be possible to distinguish between these solutions (they lack practical identifiability). Considering that the change in pool size simulated in these solutions (3-fold) was far greater than we observed for our actual apoC-III study (far less than 3-fold), we therefore feel justified in using a steady-state model for apoC-III kinetics. The apoC-III system may not actually be at a true steady state, but due to the lack of practical identifiability it would not be possible to resolve a more complicated non-steady state model. In summary, we are confident that our calculated
production rates are valid and are not affected by the small perturbations of steady state that occurred in this study.

**Statistics**

Results are presented as mean ± SEM. Paired t-test was used to compare results from the Intralipid/heparin infusion and the saline control studies. ANOVA with post-hoc Tukey’s test was used for the n=5 participants that underwent all three studies (saline, Intralipid plus heparin and glycerol infusion studies). Since no differences for any of the parameters of interest were found between the saline and glycerol infusion studies, all further statistical analyses focused exclusively on the Intralipid/heparin vs saline infusion studies. Two-way ANOVA for repeated measures was used to analyze the kinetic experiments and was performed using SAS software (Version 8.0, Cary, NC, USA). All other analyses were performed with the SPSS version14. Sample size calculation was using the gpower3 software.
References for online data supplement.


Legend to Figure S1. (A) Multicompartmental model describing TRL apoC-III₁ tracer kinetics. Compartment 1 represents the plasma amino acid precursor pool, compartment 2 is an intracellular delay compartment, which accounted for the synthesis, assembly and secretion of apoC-III, and compartment 3 is the plasma lipoproteins. Subjects were in steady-state during the kinetics study so FCR was equivalent to fractional synthetic rate, (B) Representative IEF gels showing clear separation of apoC-III₁, (C) Isotopic enrichment of TRL apoC-III with deuterium-labeled leucine. Representative enrichment curves are expressed as tracer to tracee ratio during the 12hr of d3-leucine infusion in one individual during constant enteral feeding.
Legend to Figure S2.

The figure illustrates the rise-to-plateau TTR in the apoC-III:

The starting steady-state solution is shown as a solid line, while the two non-steady state solutions are shown at discrete time points. Both non-steady state solutions show a rise toward the same TTR plateau. The time course for change in pool size in the two non-steady state solutions was identical (not shown); thus, it would not be possible to distinguish between these non-steady state solutions even if time-dependent values for apoC-III concentration were included in the model.