Online Data Supplement

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\(^1\). 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)\(^2\), stained with Coomasie blue stain and destained with 10% acetic acid. The band corresponding to the major isoform of apoC-III, apoC-III\(_1\), 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-butyldimethyl-N-methyltrifluoracetamide; Sigma-Aldrich) mixture and incubating 30 min at 70°C\(^3\) 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 multicompartamental 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. 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. Kinetic 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.