

VLDL-TG kinetics: a dual isotope study for quantifying VLDL-TG pool size, production rates, and fractional oxidation in humans

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Sørensen LP, Gormsen LC, Nielsen S. VLDL-TG kinetics: a dual isotope study for quantifying VLDL-TG pool size, production rates, and fractional oxidation in humans. *Am J Physiol Endocrinol Metab* 297: E1324–E1330, 2009. First published September 22, 2009; doi:10.1152/ajpendo.00366.2009.—Very-low-density lipoproteins (VLDLs) are large, complex particles containing both surface proteins (e.g., ApoB100) and core lipids, e.g., cholesterol and triglycerides (TG). Whereas ApoB100 kinetics have been thoroughly studied, accurate measurement of VLDL-TG kinetics have proven difficult due to either complex mathematics or laborious procedures. The present study was therefore designed to measure VLDL-TG kinetics by dual isotope *ex vivo* labeled VLDL-TG tracers and well-established kinetics equations (bolus injection or the primed continuous infusion). Ten healthy Caucasian men [age, 23 ± 3 yr old (mean \pm SD); body mass index, 24.7 ± 1.3 kg/m²] were included in the study. VLDL-TG rate of appearance (Ra) was measured using a dual-tracer technique (^{9,10}-³H]-labeled VLDL-TG and [¹-¹⁴C]-labeled VLDL-TG) to allow comparison of various bolus decay curve fits with the Ra obtained by the primed continuous infusion (PCI; considered the gold standard). In addition, VLDL-TG fatty acid oxidation was measured as ¹⁴CO₂ in exhaled breath, using the hyamine trapping technique. Following a bolus injection, tracer decay was better described by a biexponential than a monoexponential fit ($r^2 = 0.99 \pm 0.01$ vs. 0.97 ± 0.04 , respectively, $P = 0.01$). VLDL-TG Ra calculated using the PCI correlated significantly with the biexponential fit ($\rho = 0.62$, $P < 0.05$), whereas this was not the case for the monoexponential fit ($\rho = -0.18$, $P =$ not significant). VLDL-TG Ra using the best fit of the bolus injection method (biexponential) was less than values obtained by the constant infusion technique [biexponential, 34.3 (range, 27.1–69.6) vs. PCI, 44.4 (range, 33.0–72.7), $P < 0.05$]. Fractional oxidation of VLDL-TG was $37.2 \pm 8.8\%$ at 240 min corresponding to 198.8 ± 55.9 kcal/day or $10.6 \pm 3.3\%$ of resting energy expenditure (REE). Our data demonstrate that VLDL-TG Ra measured by a biexponential fit to a bolus decay curve correlates well with VLDL-TG Ra measured by a primed continuous infusion, and therefore that a “second” peripheral VLDL-TG compartment with rapid exchange of TG exists. VLDL-TG volume of distribution is therefore greater than previously anticipated. Finally our data supports that VLDL-TG contributes quantitatively to REE.

lipoproteins; tracer kinetics; methods; margination

ELEVATED LEVELS OF CIRCULATING TRIGLYCERIDES (TG) are by now an established risk marker for cardiovascular disease (11). In clinical practice, TG concentration is measured in the morning after a short-term fast (~ 12 h), at which point the majority of TG circulate in very-low-density-lipoproteins (VLDLs) with only a smaller fraction in other lipoprotein particles (e.g., IDL and LDL). VLDLs are complex particles, consisting of an outer

layer of hydrophilic scaffolding and receptor proteins with a core of lipids, such as TG and cholesterol. Although it is relatively simple to measure static TG concentrations, it was due to the complexity of the VLDL particle, proven difficult to apply traditional tracer techniques to accurately measure production and clearance of VLDL particle subcomponents, as reviewed previously by Magkos and Sidossis (17).

Several tracer methods have been developed based on administration of a TG precursor, which is eventually incorporated into VLDL-TG. A common approach is to inject a bolus of tracer (i.e., labeled palmitate or glycerol) and determine VLDL-TG kinetics from the subsequent decay of labeled VLDL-TG. Traditionally, data have been modeled using a monoexponential approach under the assumption that VLDL particles are confined to the bloodstream. VLDL-TG production rates have then been calculated by multiplying the fractional catabolic rate with VLDL-TG pool size estimated as VLDL-TG concentration times plasma volume (13, 16, 21). However, precursor labeling is complicated by complex precursor kinetics and tracer recycling, which can influence the calculation of VLDL-TG turnover. In addition, there are indications that the best fit to the decay curve is not always monoexponential (7). To account for this, various more or less complicated compartmental models have been proposed (4, 8, 18, 20, 28). Although compartmental modeling is a useful tool and allows for use of simple and commercially available tracers, there are indications that estimates of VLDL-TG production rates and pool size estimates calculated by this method are significantly lower than those obtained by measuring splanchnic balances (2, 3, 9), considered the most accurate measurement of VLDL-TG production.

A conceptually simpler approach than multicompartmental modeling is administration of a “true” VLDL-TG tracer, i.e., whole VLDL particles containing a lipid tracer (e.g., TG containing a stable or unstable isotope), with subsequent calculation of VLDL-TG kinetics using the traditional tracer dilution technique. In theory, as no precursor kinetics are involved, study protocols of shorter duration are sufficient to determine kinetic parameters of interest, and tracer recycling problems are circumvented. This approach has been used with some success by Sidossis et al., (23) who developed a method based on *in vivo* labeling of VLDL-TG, plasmapheresis and subsequent primed continuous reinfusion. The method is intuitively very appealing and elegant, but tracer production involving plasmapheresis is laborious and therefore not practically applicable in all labs.

In our lab, we have developed an alternative method to produce a “true” VLDL-TG tracer based on *ex vivo* labeling with subsequent reinfusion (5, 6). The TG moiety of the VLDL particles is labeled by incorporation of triolein molecules in which fatty acids are labeled with either ¹⁴C or ³H or both. In

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addition to calculation of VLDL-TG kinetic parameters of interest, the method allows, by tracing the VLDL-TG associated fatty acids, the study of their metabolic fate, i.e., to what extent they are oxidized or deposited in adipose tissue (7).

The purpose of the present study was therefore to use our ex vivo labeled VLDL-TG tracer ([9,10-³H]triolein VLDL-TG and [1-¹⁴C]triolein VLDL-TG) to 1) establish, by noncompartmental analysis, the best fit (mono- or biexponential) to a bolus injection decay curve, 2) calculate basal VLDL-TG production rates and pool size using both primed continuous infusion and bolus injection and to compare these values with values obtained by other techniques, and 3) estimate the fraction of VLDL-TG fatty acid channeled toward oxidation.

RESEARCH DESIGN AND METHODS

The study protocol was approved by the local ethics committee and informed consent was obtained from all participants.

Subjects

Ten Caucasian men (age, 23 ± 3 years; body mass index, 24.7 ± 1.3 kg/m²) were recruited for this study. All were in good health and had normal physical examinations and laboratory evaluations, and none used any medications. In addition, all were nonsmokers and abstained from strenuous exercise 72 h before the study day.

Experimental Protocol

One week prior to the study day volunteers visited the Clinical Research Laboratories in the morning after an overnight fast. A physical examination was performed, and blood samples were obtained for screening purposes (lipid profile, HbA1c, liver and kidney function, complete blood count) and for subsequent ex vivo labeling and isolation of VLDL-TG as described below.

At the study day, subjects attended the study site at 0700 ($t = -60$ min) in the morning after an overnight fast. Catheters were placed in an antecubital vein for infusions and a contralateral heated hand vein for blood sampling. At 0800 ($t = 0$ min), after baseline blood and breath sampling, the [9,10-³H]VLDL-TG tracer and 33% of the [1-¹⁴C]VLDL-TG tracer was infused as a bolus, and a constant infusion with the remaining 67% of the [1-¹⁴C]VLDL-TG tracer was started. Blood samples were drawn to determine VLDL-TG specific activity (SA) at $t = 0, 5, 10, 30, 60, 120, 180, 210,$ and 240 min and metabolite and insulin concentrations every 60 min from $t = 0$. Breath samples were also obtained every 60 min from $t = 0$. Indirect calorimetry (Deltatrac monitor; Datex Instruments) was performed for 30 min from $t = -30$ min and 10-min measurements were performed every 60 min from $t = 60$. After the blood sampling at 240 min the catheters were removed and the participants were discharged.

VLDL-TG Tracer Preparation

One week prior to the study day, an 80-ml blood sample was obtained under sterile conditions. Plasma was separated by centrifugation (3,600 rpm at 4°C for 30 min) and each half portion of plasma was transferred to sterile test tubes containing either 120 μCi [9,10-³H]triolein or 30 μCi [1-¹⁴C]triolein (PerkinElmer) dissolved in 300 μl ethanol (Merck). Plasma containing labeled triolein was then sonicated in a cell incubator at 37°C for 6 h. To isolate the labeled VLDL particles, labeled plasma was transferred to sterile 6-ml centrifuge tubes (Beckman Instruments), covered with a saline solution ($d = 1.006$ g/ml), and centrifuged (Ti 50.3 rotor; Beckman Instruments) for 18 h at 40,000 rpm and 4°C. The supernatant containing the labeled VLDL fraction was obtained by a sterile glass Pasteur pipette, and finally the solutions were filtered (Filtropur S 0.2; SARSTEDT). A 100-μl sample of each of the solutions was transferred to a scintillation vial, scintillation fluid (Optiphase HiSafe 2; PerkinElmer)

was added, and ¹⁴C and ³H activity was measured by dual-channel liquid scintillation counting (Wallac 1409; PerkinElmer) to a < 2% counting error. The total amount of ¹⁴C and ³H activity in the infused tracers was 2.9 (0.6–4.9) and 13.6 (3.5–36.2) μCi [means (range), respectively]. Samples were tested for bacterial growth to ensure sterility, and the solutions were stored at 4°C until use (<1 wk).

Plasma VLDL-TG Concentration and SA

VLDL particles were isolated from ~3 ml of each plasma sample by ultracentrifugation as described above. The supernatant containing the VLDL fraction was obtained by slicing the tube ~1 cm from the top, using a tube slicer (Beckman Instruments), and transferred to a scintillation vial. A 300-μl sample was analyzed for TG concentration. The volume of the remaining solution was calculated by weighing the vial both empty and with the solution. Scintillation fluid was added to the vial, and ¹⁴C and ³H activity was measured by dual-channel liquid scintillation counting to a < 2% counting error.

Breath CO₂ SA

To obtain breath samples, subjects expired into breath bags (IRIS-breath-bags; Wagner Analysen Technik). The exhaled air was passed through a solution containing 0.5 ml hyamine hydroxide in 1 M methanol (Zinsser Analytic, Berkshire, UK), 2 ml of 96% ethanol (BDH Laboratory Supplies, Poole, UK), and two drops of thymolphthalein (Sigma-Aldrich, St. Louis, MO) in a scintillation vial. A color change (blue to clear) occurred when exactly 0.25 mmol CO₂ was trapped in the solution. Scintillation fluid was added to the vial, and ¹⁴CO₂ activity was measured by liquid scintillation counting to a < 2% counting error.

Sample Collection and Assays

All blood samples were placed on ice and separated as quickly as possible by centrifugation (3,600 rpm at 4°C for 10 min). Aliquots of plasma (3 ml) were stored at 4°C for isolation of VLDL upon completion of the examination as described above. The remaining samples were stored at -20°C for later analysis. TG concentrations were analyzed using a COBAS Fara II (Hoffmann-La Roche). Serum insulin concentrations were measured with an immunoassay (DAKO). Serum free fatty acid (FFA) concentrations were determined by a calorimetric method using a commercial kit (Wako Pure Chemical Industries).

Calculations

VLDL-TG Ra: primed constant infusion. VLDL-TG Ra (μmol/min) was calculated by dividing the infusion rate (F) by the plateau (SA)

$$\text{VLDL-TG Ra} = \frac{F}{SA}$$

VLDL-TG Ra: bolus infusion. The VLDL-TG SA vs. time data following the bolus of tracer was analyzed by nonlinear least-squares regression analysis using a mono-, a bi- and a triexponential decay model. A triexponential equation did not give a markedly better fit than a biexponential equation and was not used for subsequent calculations. Curve fitting analyses were done by means of the computer program SigmaPlot version 10.00 for Windows (Systat Software). Using the monoexponential model, VLDL-TG SA at any time point can be described by

$$SA(t) = A_1 \times e^{(-B_1 \times t)}$$

where B1 is the decay constant and A1 denotes the theoretical intercept constant of the slope. Likewise, using the biexponential model, VLDL-TG SA at any time point can then be described by

$$SA(t) = A_1 \times e^{(-B_1 \times t)} + A_2 \times e^{(-B_2 \times t)}$$

where B1 and B2 are decay constants of the early and late phase, respectively, and A1 and A2 denote theoretical intercept constants of the early and late slopes. VLDL-TG Ra ($\mu\text{mol}/\text{min}$) was calculated by dividing the dose by the area under the curve of SA vs. time following the bolus of tracer. This can be expressed using an integral approach or in terms of the parameters of the curve fits.

Integral approach

$$\text{VLDL-TG Ra} = \frac{\text{Dose}}{\int_0^{\infty} SA(t) dt}$$

Monoexponential fit

$$\text{VLDL-TG Ra} = \frac{\text{Dose}}{A_1/B_1}$$

Biexponential fit

$$\text{VLDL-TG Ra} = \frac{\text{Dose}}{A_1/B_1 + A_2/B_2}$$

VLDL-TG pool size: bolus infusion. The size of the apparent pool (μmol) was calculated by dividing the size of the bolus by the initial specific activity.

$$\text{VLDL-TG pool size} = \frac{\text{Dose}}{SA(0)}$$

SA(0) was calculated by inserting the value of $t = 0$ in the exponential equations obtained by curve fitting.

VLDL-TG fatty acid oxidation: primed constant infusion. Fractional oxidation (%) of the infused [$1\text{-}^{14}\text{C}$]VLDL-TG was calculated as follows.

$$\text{fractional VLDL-TG oxidation} = \frac{{}^{14}\text{CO}_2\text{SA} \times \dot{V}\text{CO}_2}{k \times \text{Ar} \times F}$$

Here, k is the volume of CO_2 at 20°C and 1 atm pressure (22.4 l/mol), Ar is the fractional acetate carbon recovery factor in breath CO_2 , and F is the tracer infusion rate. Sidossis et al. (22) has previously calculated Ar to be 0.56 for resting conditions. The total VLDL-TG oxidation rate ($\mu\text{mol}/\text{min}$) was calculated as VLDL-TG oxidation rate = fractional VLDL-TG oxidation \times VLDL-TG Ra.

VLDL-TG fatty acid oxidation rate ($\mu\text{mol}/\text{min}$) was calculated by multiplying VLDL-TG oxidation rate by 3 because of the 1:3 molar ratios. To calculate energy production (kcal/day) from VLDL-TG fatty acids, the rate of VLDL-TG fatty acid oxidation was converted to its weight equivalent using the molecular weight of 282 g/mol oleic acid and multiplying the caloric density of by 9.1 kcal/g and 1,440 min/day.

Statistics

All data are expressed as means \pm SD unless stated otherwise. Variables that were not normally distributed were log transformed before statistical processing. Between-group differences were analyzed using Student's t -test or the Mann-Whitney two-sample test. Correlations were evaluated by Pearson's r or Spearman's ρ test. Differences were considered significant at $P < 0.05$.

RESULTS

Hormones and Metabolites

As expected, concentrations of TG, VLDL-TG, FFA, insulin, and glucose remained stable during the 240-min examination period (Fig. 1).

VLDL-TG Ra: Primed Constant Infusion

After an initial decrease in SA following the priming dose, VLDL-TG SA steady state was reached after 30–60 min (Fig. 2A). VLDL-TG Ra was calculated based on the plateau SA during the time period from 60 min and onward as steady state was reached in all subjects. The VLDL-TG Ra was calculated to 44.4 (33.0–72.7) $\mu\text{mol}/\text{min}$ [median (range)].

VLDL-TG Ra: Bolus Infusion

The tracer disappearance curves after bolus infusion (Fig. 2B) were fitted to a mono-, a bi- and a triexponential decay function. The biexponential approach yielded a markedly better fit than the monoexponential approach assessed by visual inspection of the VLDL-TG SA vs. time data (Fig. 2C) as well as the adjusted r^2 [biexponential, 0.996 (range, 0.998–0.981) vs. monoexponential, 0.976 (range, 0.999–0.855), $P < 0.03$]. The triexponential approach did not give a markedly better fit than the two-compartment model as assessed graphically (data not shown) or as the adjusted r^2 ($P = 0.734$) and was not used for subsequent calculations.

The absolute VLDL-TG Ra values were [VLDL-TG Ra in $\mu\text{mol}/\text{min}$: biexponential, 34.3 (range, 27.1–69.6) vs. monoexponential, 42.9 (range, 30.5–68.0), $P =$ not significant]. Thus, although the mean values from the monoexponential fit to the bolus curve appeared to be more in agreement with the values obtained by the constant infusion, results from individual subjects fit better with the biexponential fit.

VLDL-TG Ra: Correlations

VLDL-TG Ra determined by primed constant infusion correlated significantly with VLDL-TG Ra determined by bolus infusion using the biexponential model ($\rho = 0.62$, $P < 0.05$) (Fig. 3A) but not using the monoexponential model ($\rho = -0.18$, not significant) (Fig. 3B). However, VLDL-TG Ra, determined by the two-exponential model, underestimated VLDL-TG Ra as determined by primed constant infusion at an average of $20.4 \pm 14.5\%$ ($P < 0.05$), and both the difference and the variability tended to get progressively larger as the average increased (Fig. 3C).

VLDL-TG Pool Size

The apparent VLDL-TG pool size was 3,093 (range: 997–4,723) μmol by using the biexponential approach.

VLDL-TG Fatty Acid Oxidation

Fractional oxidation of the infused VLDL-TG tracer was $37.2 \pm 8.8\%$ at 240 min (Fig. 4) equivalent to 17.9 ± 5.0 μmol triolein/min, thus corresponding to 198.8 ± 55.9 kcal/day or $10.6 \pm 3.3\%$ of REE. However, steady state was not reached, and presumably the fractional oxidation is underestimated in these healthy subjects.

DISCUSSION

In the present study, simultaneous bolus infusion of ex vivo labeled [$9,10\text{-}^3\text{H}$]triolein VLDL-TG and primed constant infusion of ex vivo labeled [$1\text{-}^{14}\text{C}$]triolein VLDL-TG was used to demonstrate, that 1) the best fit to the decay curve following a bolus injection of labeled VLDL-TG is biexponential; 2) that the VLDL-TG production rates thus obtained are in good

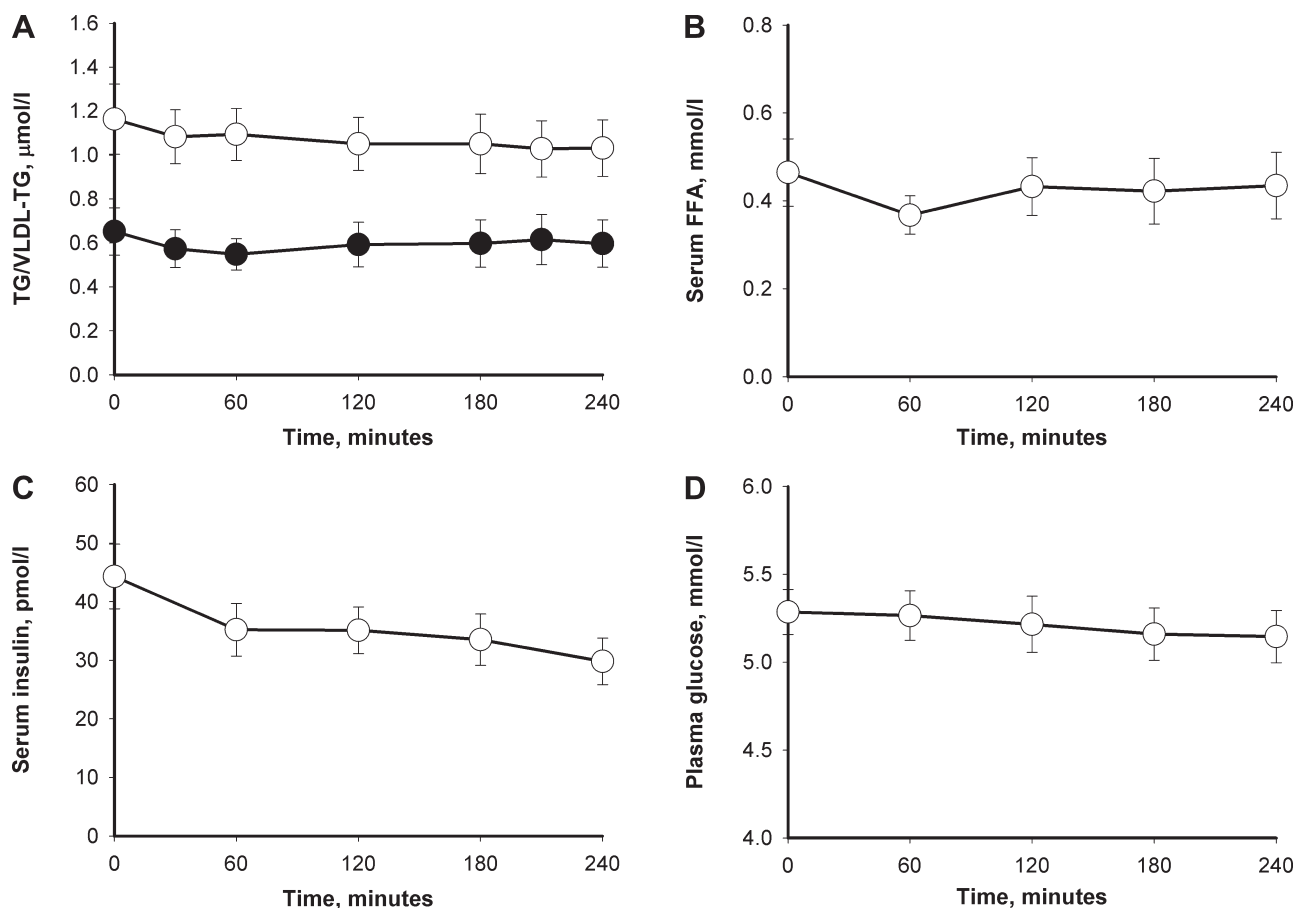


Fig. 1. Time course for concentrations of plasma triglycerides (TG; \circ) and very-low-density lipoproteins (VLDL)-TG (\bullet) (A), serum free fatty acid (FFA; B), serum insulin (C), and plasma glucose (D) during the 240-min examination period. Data are means \pm SE.

agreement with those obtained by a primed continuous infusion; 3) that basal VLDL-TG production rates and pool size are greater than previously reported, and 4) that VLDL-TG oxidation accounts for a significant proportion of REE.

Traditionally, VLDL particles have been thought to be confined to a single compartment, namely the bloodstream. Under this assumption, tracer decay curves following a bolus injection have been fitted to simple monoexponential decay function and the decay constant (FCR) of the VLDL-TG pool has then been calculated or eyeballed as the slope of the curve. However, we recently studied the impact of body composition on VLDL-TG kinetics in obese women (7) and found that the best fit to observed decay curves was biexponential rather than monoexponential. Similar decay patterns have been reported by others (12). Using a true *ex vivo* labeled VLDL-TG tracer with early sampling (from 5 min and onward) from the bolus injection, the current study confirms that labeled VLDL-TG decays biexponentially when no precursor label is present.

In addition to allowing early sampling and precise characteristics of the early part of the decay curve, the tracer approach applied in the present study allows calculation of kinetics parameters of interest without the assumptions inherent to methods based on *in vivo* labeling and mathematical modeling. The resulting VLDL-TG production rates, expressed in $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ (means \pm SD), for reasons of comparison, were 665 ± 233 using bolus infusion

and 840.0 ± 247 using primed constant infusion. The values are in agreement with or higher than values from studies in healthy, lean men using other model-independent methods e.g., primed constant infusion technique (506 ± 219) (23), or splanchnic balances (286 ± 87) (9), (570 ± 222) (2) or (757 ± 468) (3) but considerably higher than those obtained using techniques relying on estimates of FCR and pool size (192 ± 88) (21) or (263 , SD unknown) (13). However, more recent studies involving use of [$^2\text{H}_5$]glycerol VLDL-TG in combination with mathematical modeling have provided estimates of VLDL-TG turnover more in agreement with model independent results, implying that [$^2\text{H}_5$]glycerol is less susceptible to tracer recycling issues than other endogenous tracers (16, 20). Data presented herein thus suggest that the kinetics following bolus infusion of VLDL-TG tracer is better described by a biexponential rather than a monoexponential decay function and indicate that the use of a monoexponential fit to the decay curve following injection of a VLDL-TG precursor fails to yield information regarding the first fast decay constant. Taken together, this underscores the importance of precise modeling and frequent sampling when using labeled TG precursors.

The apparent kinetic heterogeneity of labeled VLDL-TG strongly suggests that a peripheral compartment with reversible exchange of TG with circulating VLDL particles exists. In this case, exchange of labeled VLDL-TG for unlabeled TG produces a second, slower exponential in the decay curve. There

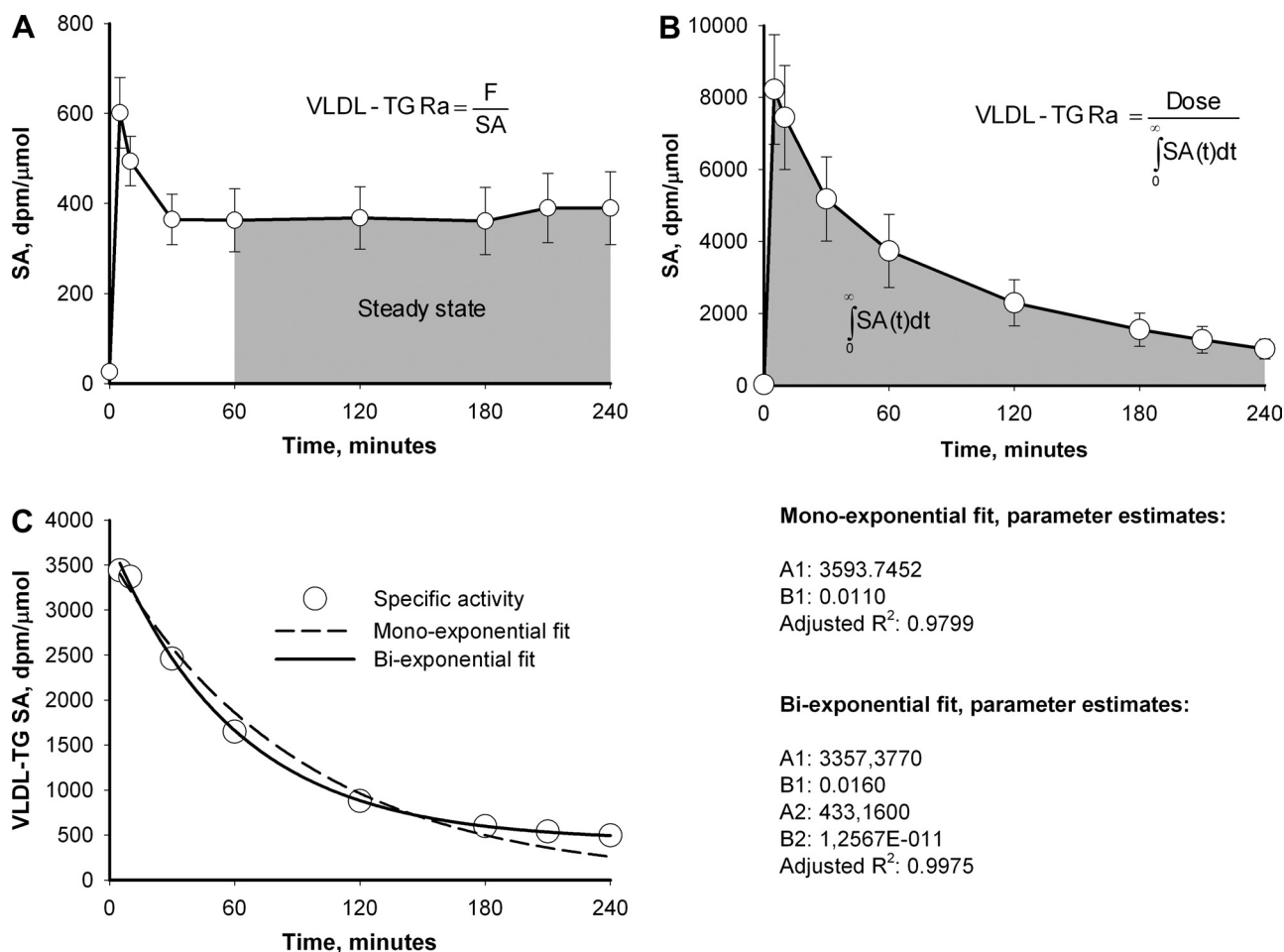


Fig. 2. Time course of plasma [^{14}C]-labeled VLDL-TG SA (dpm/ μmol) during primed constant infusion (A) and plasma [^3H]-labeled VLDL-TG SA (dpm/ μmol) after bolus infusion (B) of ex vivo labeled VLDL-TG tracer in all subjects. Data are means \pm SE. Comparison of monoexponential and biexponential fit to the VLDL-TG SA vs. time curve in a representative subject (C) with estimates of decay and intercept constants and adjusted R^2 . Ra, rate of appearance; A1 and A2, theoretical intercept constants of the early and late slopes, respectively; B1 and B2, decay constants of the early and late phase, respectively; F, infusion rate; SA, plateau.

are several candidate pools with which VLDL-TG may equilibrate. First, on the basis of a biexponential decay of labeled lipoproteins, Karpe et al. (12) have proposed that ApoB containing lipoproteins are marginated in a quantitatively important fashion, i.e., that TG-rich lipoproteins attach reversibly to the endothelium of healthy subjects. Physiologically relevant margination of TG-rich lipoproteins has later been confirmed by others (19). Circulating TG-rich lipoproteins represent an important source of energy and serve as a means of transporting lipid energy to peripheral tissues. In the capillary beds, lipoproteins are attached to the endothelium, and TG is hydrolyzed by lipoprotein lipase-releasing FFAs for oxidation in energy-consuming tissues. It is therefore reasonable to assume that a substantial part of lipoproteins (labeled and unlabeled) will be attached to the endothelium at any given time. In line with this, it has been estimated by several groups (12, 19) that ApoB containing lipoprotein volume of distribution is almost twofold larger than estimated plasma volume, rendering the notion that VLDL particles are less likely confined to the bloodstream. Second, although cholesteryl ester transfer protein-mediated transfer of triglycerides for cholesterol between VLDL and high-density lipoproteins is often thought of as a

one-way process, in vitro studies have suggested that this transfer is frequently a homoexchange, i.e., a TG molecule is exchanged for a TG molecule (1, 14). TGs from other lipoprotein fractions may therefore, in some cases, be transported back into the VLDL fraction, further expanding its apparent pool size. In agreement with this, the calculated apparent VLDL-TG pool size ($3,093 \pm 1,285 \mu\text{mol}$) observed in the present study was significantly greater ($P < 0.001$) than the VLDL-TG pool estimated from plasma volume and VLDL-TG concentration ($2,251 \pm 1,059 \mu\text{mol}$). Other studies involving labeled VLDL (26) and LDL also indicate that the lipoprotein apparent volume of distribution does not equate with plasma volume. Thus, 99m-technetium-labeled LDL particles rapidly attach to the endothelium of large blood vessels and are sequestered in the liver as can be visualized with a gamma camera (15). These observations have implications for subsequent calculation of VLDL-TG kinetics. Thus, if the reversible exchange (micro rate constants) of TG between circulating VLDL and endothelium-bound VLDL is slow, isotopic equilibrium may not be reached during a short primed constant infusion protocol. In this case, the primed constant infusion will underestimate the "true" steady-state SA of the total VLDL-TG pool, resulting in

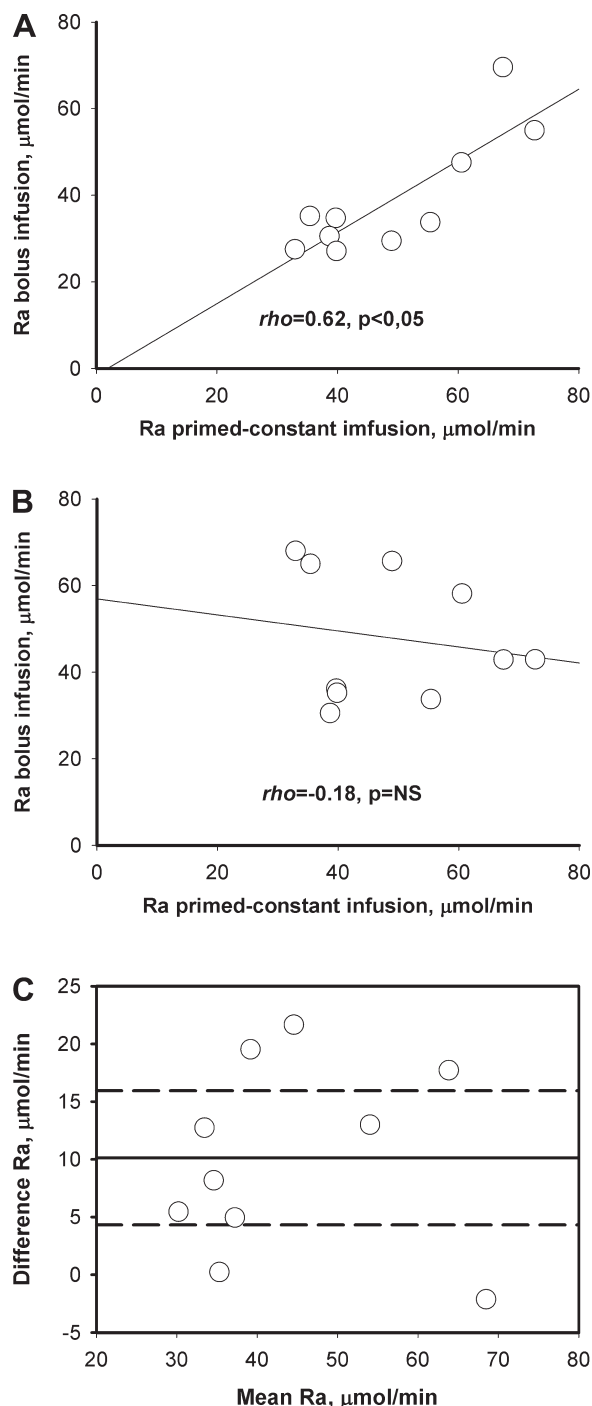


Fig. 3. Relationship between VLDL-TG Ra ($\mu\text{mol}/\text{min}$) as determined by primed constant infusion and bolus infusion of ex vivo labeled VLDL-TG tracer using a biexponential model (A) or a monoexponential model (B). Bland-Altman plot illustrating the agreement between-TG Ra as determined by primed constant infusion and bolus infusion using a biexponential model (C). The full line and the dotted lines represent the mean of the difference and 95% confidence intervals, respectively.

overestimation of VLDL-TG Ra. However, based on the bolus decay of labeled VLDL presented in this paper, equilibrium between VLDL-TG in the bloodstream and endothelium bound is obtained within hours, enabling precise estimates of VLDL-TG Ra using a 240-min primed constant infusion.

Although our VLDL-TG Ra estimates obtained by bolus injection and constant infusion were in good agreement, we did observe a systematic difference of $\sim 20\%$ as depicted in the Bland-Altman plot. This discrepancy may be caused by tracer recycling. If a significant proportion of the VLDL-TG tracer following bolus infusion is taken up by the liver, stored internally, and later secreted as VLDL-TG, it will cause the appearance of a second, slower exponential. As a result, the tail of the decay will increase, and, as the estimation of decay constants and, therefore also, calculation of VLDL-TG Ra is strongly influenced by the tail of the decay, this may result in underestimated VLDL-TG Ra. This tracer recycling problem represents a minor problem in constant infusion protocols, since recycled labeled VLDL-TG contributes a minute proportion to steady-state SA when final VLDL-TG Ra is calculated. Tracer recycling is a common problem in bolus injection studies and various correction factors (27) or alternative fits to bolus decay curves (10) are often introduced to circumvent this problem. When a bolus injection of ex vivo labeled VLDL-TG is deemed appropriate (e.g., in biopsy studies), we therefore suggest correcting calculated values by a factor of 1.2.

It is obvious to question why the observed kinetic heterogeneity is not evident using precursor labeling. In methods based on precursor labeling, FCR is determined by fitting a straight line function to the experimental data under the assumption that the precursor is rapidly taken up by and secreted by the liver as VLDL-TG. However, due to complex precursor kinetics the data are inherently nonlinear. Because of this, and due to potential tracer recycling, kinetic heterogeneity may not be evident using this approach.

An additional purpose of the present study was to examine whether fractional and total VLDL-TG oxidation rates can be estimated using the novel approach of a primed constant infusion of ex vivo [^{14}C]triolein labeled VLDL-TG in combination with measurements of CO_2 production and $^{14}\text{CO}_2$ SA in expired air. Only a few other studies (24, 25) have attempted to measure VLDL-TG oxidation, and estimates vary greatly. During the labeled VLDL-TG infusion, $^{14}\text{CO}_2$ SA rapidly increased, and fractional oxidation reached 40% after 240 min, corresponding to $>10\%$ of the REE. However, we did not reach steady state in all subjects, and fractional oxidation was therefore most likely underestimated. Using a biexponential fit

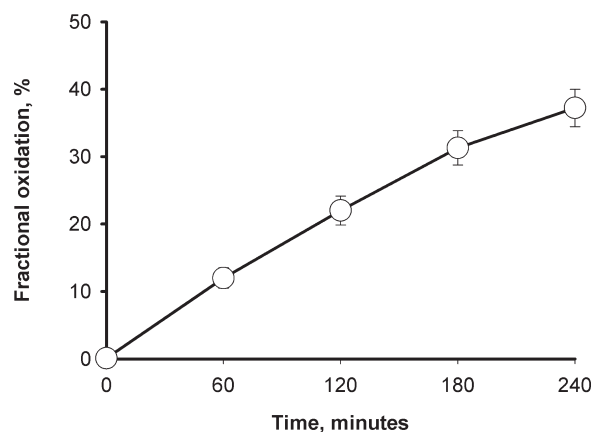


Fig. 4. Time course of the fractional oxidation (%) of infused [^{14}C]-labeled VLDL-TG during primed constant infusion of ex vivo labeled VLDL-TG tracer. Data are means \pm SE.

to tracer decay curves we recently reported that VLDL-TG oxidation accounted for ~20% of REE in lean and obese women, and, taken together, these observations indicate that circulating TG constitute a significant energy source in fasting humans. Further studies are required to describe the proportional fat oxidation stemming from circulating FFA, VLDL-TG, and intramyocellular TG stores and whether VLDL-TG oxidation rates are altered significantly during altered energy requirements (e.g., exercise).

In summary, we herein present data supporting the notion that labeled VLDL-TG decays biexponentially and thus that a second peripheral VLDL-TG compartment may exist, that earlier model-dependent VLDL-TG production and pool size estimates may be too low, and that VLDL-TG contributes to REE in a quantitatively significant fashion.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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