A New Combined Multicompartmental Model for Apolipoprotein B100 and Triglyceride Metabolism in VLDL Subfractions

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ABSTRACT

The use of stable isotopes in conjunction with compartmental modeling analysis has greatly facilitated studies of the metabolism of the apoB-containing lipoproteins in humans. The aim of this study was to develop a multicompartment model that allows to simultaneously determine the kinetics of apoB and TG in VLDL₁ and VLDL₂ after a bolus injection of $^{2}H_{3}$-leucine and $^{2}H_{5}$-glycerol, and to follow the catabolism and transfer of the lipoprotein particles. Here, we describe the model and present the results of its application in a fasting steady-state situation in 17 subjects with lipid values representative of a Western population. Analysis of the correlations showed that plasma TG was determined by the VLDL₁ and VLDL₂ apoB and TG FCR. Furthermore, the model showed a linear correlation between VLDL₁ TG and apoB production. A novel observation was that VLDL-TG entered the circulation within 21 min after its synthesis, whereas VLDL-apoB entered the circulation after 33 min. These observations are consistent with a sequential assembly model of VLDL and may suggest that the TG is added to a primordial apoB-containing particle in the liver.

Key words: • VLDL • apolipoprotein B • triglycerides • compartmental modeling • kinetics
• stable isotope • assembly
INTRODUCTION

Regulation of the metabolism of VLDL subfractions has been an area of active interest that received fresh impetus from the introduction of stable isotope–based techniques in the late 1980s (1,2). The use of tracer models has generated direct information on lipoprotein synthetic rates, which previously could only be inferred from the turnover of radiolabeled lipoproteins. One common approach is to inject a bolus of radioactive tracer, such as \( {\text{\(^3\)}}H, {\text{\(^{14}\)}}C \) glycerol, and determine the subsequent monoexponential slope of the decline in plasma VLDL–specific radioactivity. A disadvantage of this approach is that it can underestimate the true VLDL turnover rate because it does not account for recycling of the injected bolus of tracer (3). Multicompartmental modeling improves the accuracy by attempting to account for tracer recycling (3-8). Such studies have revealed that VLDL\(_1\) apoB100 production and VLDL\(_2\) apoB100 production are independently regulated (9-11), indicating that regulatory steps in the assembly of VLDL govern the lipid content of the secreted particles. However, it is still unclear how the liver regulates the TG content of VLDL particles to produce large VLDL\(_1\) or small VLDL\(_2\). VLDL assembly is thought to involve at least two steps in which nascent VLDL particles are formed and then TG is added, resulting in larger particles (12,13).

Several studies have analyzed VLDL TG turnover kinetics using stable isotopically labeled glycerol or palmitate tracers and mathematical modeling. However, VLDL subclasses were not analyzed in those studies, and VLDL apoB was not included in the models (3,14,15). To enhance our understanding of the pathways leading to VLDL\(_1\) and VLDL\(_2\) and of the metabolic fate of these particles, we developed for the first time a multicompartmental mathematical model that allows the kinetics of TG and apoB100 in VLDL\(_1\) and VLDL\(_2\) to be simultaneously assessed after a bolus injection of glycerol and leucine. Here, we describe the model and present the results of its application in 17 subjects with lipid values representative of a Western population.
METHODS

Subjects and materials—Seventeen healthy subjects were recruited for the study. The purpose, nature, and potential risks of the study were explained to all subjects before their written consent was obtained. The study protocols were approved by the ethical committee of the Helsinki University Central Hospital. All materials were from Sigma Chemical Company (Poole, Dorset, UK) unless otherwise stated.

ApoB and TG turnover protocol—All subjects were admitted at 7:30 a.m. to the metabolic ward of the Helsinki University Central Hospital after a 12-h overnight fast. An indwelling cannula was inserted into an antecubital vein for infusions. A second cannula was inserted retrogradely into a heated hand vein to obtain arterialized venous blood for sampling. A saline infusion was started. Thirty minutes later, leucine (5,5,5-D3), 7 mg/kg body weight (bw), and glycerol (1,1,2,3,3-D5), 500 mg (Isotec, Miamisburg, OH), were injected as a bolus. For measurement of free $^2\text{H}_3$-leucine concentration in plasma, blood samples were taken before the tracer injection and at 2, 4, 6, 8, 10, 12, 15, 20, 30, and 45 min and 1, 2, 3, 4, 6, and 8 h. For measurement of $^2\text{H}_3$-leucine and $^2\text{H}_5$-glycerol in VLDL$_1$ and VLDL$_2$, blood samples were taken before the injection of tracers and at 15, 30, 45, 60, 75, 90, 120, and 150 min and 3, 4, 5, 6, 7, and 8 h. The particle composition and apoB mass of the VLDL$_1$ and VLDL$_2$ fractions were determined 30 min before and 0, 4, and 8 h after the injection. The subjects continued to fast until 5 p.m., when the last blood sample was taken.

Isolation of lipoproteins—VLDL$_1$ and VLDL$_2$ were isolated from 8.4 ml of plasma as described (16). The apoB and TG pool sizes were analyzed from samples obtained at 0, 4, and 8 h and prepared as described (16). Pool sizes for apoB and TG were calculated as the product of plasma volume (4.5% of bw) and the plasma concentration of apoB and TG in VLDL$_1$ and VLDL$_2$. The leucine content of the apoB pool was calculated from the apoB amino acid residue composition. The glycerol content was calculated from the TG concentration using a molecular weight of 885 g/mol for TG and 92 g/mol for glycerol and assuming that one mole of TG equals one mole of glycerol.

Biochemical analyses—TG and cholesterol concentrations in total plasma and in all lipoprotein fractions were determined by automated enzymatic methods (Cobas Mira analyzer, Hoffman-La Roche, Basel, Switzerland). ApoB was analyzed in the plasma lipoprotein fractions as described (17). Serum
glucose, insulin, free fatty acids, and alanine transaminase were analyzed as described (18). Protein concentrations in lipoprotein fractions were measured by the method of Kashyap et al. (19).

**Determination of leucine enrichment in apoB**—The samples were precipitated with isopropanol, delipidated with ethanol-diethyl ether, dried, and hydrolyzed with 6 M HCl at 110°C for 22–24 h (16). The samples were then prepared for analysis of leucine enrichment (20), and the $^2$H$_3$-leucine enrichments in protein hydrolysates and plasma amino acids were performed as described (21). Enrichments were determined by gas chromatography mass spectrometry (GC/MS) with a quadrupole GC/SM instrument (MD 800, Fisons, Manchester, UK).

**Determination of glycerol enrichment in TG**—The samples were precipitated with isopropanol and delipidated twice with ethanol-diethyl ether as described (20). The supernatants were combined, and the volume was increased to 20 ml with isopropanol. To remove phospholipids, 2 g of activated zeolite (product no. 96096, Fluka Biochemika, Buchs, Switzerland) was added to each tube and mixed for 20 min. After centrifugation, the supernatants were evaporated under N$_2$ at 80°C. Isopropanol (1 ml) was added to each tube, transferred into a 1.5 ml vial, and dried on a heating block at 80°C. The glycerol samples were stored at –80°C. The amount of diacylglycerol and monoacylglycerols not extracted in the supernatant was not determined. This has been reported to be a minor contaminant, accounting for 2–10% of the total plasma triglyceride (22). Immediately before analysis, the glycerol extracts were saponified with 250 µl of 2% KOH in ethanol, incubated at 60°C for 2 h, and dried under N$_2$ at 70°C for 2 h.

In three subjects, glycerol was isolated as described by Patterson et al. (23). Briefly, plasma proteins were precipitated with ice-cold acetone, equal volumes of hexane and water were added to the supernatant, and the upper phase (hexane) was dried in a centrifugal evaporator.

Glycerol was derivatized to its 1,2,3 triacetate ester by adding equal volumes of pyridine and acetic anhydride (24). Enrichments were determined with a quadrupole GC/MS instrument (Trio-1000, Fisons, Manchester, UK) under electron ionization conditions within 24 h after saponification. Samples (1–3 µl) were injected automatically into a 30 m x 0.25 mm (I.D.) x 0.25 µm DB5MS capillary column fitted with a 2-m plain silica guard column (J&W, Folsom, CA), which was run isothermally at 195°C, using a split ratio of 1:50, helium as the carrier gas, and a head pressure of 70 kPa (10 psi). The glycerol
derivative eluted at approximately 3.5 min. Under these conditions, the derivative fragments between carbons 1 and 2 or 2 and 3 of the glycerol backbone resulted in the formation of two symmetrical fragments of m/z 145 and two symmetrical fragments of m/z 73 for the unlabeled derivative (24).

The penta-deuterated derivative formed a tri-deuterated fragment at m/z 148 and a bi-deuterated fragment at m/z 75. Monitoring the larger fragment (m/z 148) allowed measurements to be made against a very low natural background, resulting in greater sensitivity than monitoring the smaller ion fragment. Ion mass fragments at m/z 147 and 148 were monitored in the selective ion recording mode. Ion peaks areas were integrated and quantified in arbitrary units with the LabBase GC/MS data management system (Fisons).

To calculate isotope enrichments, the average value of the m/z 147:m/z 145 ratio was determined in the baseline sample. This value was multiplied by the m/z 148:m/z 147 ratio, and the resulting m/z 148:m/z 145 values were expressed as molar percent excess (mpe) by the following formula:

\[
\text{mpe} = \left( \frac{\text{IR}_t - \text{IR}_b}{1 + (\text{IR}_t - \text{IR}_b)} \right) \times 100
\]

where \( \text{IR}_t \) is the m/z 148:m/z 145 peak area ratio for the enriched sample at time \( t \) and \( \text{IR}_b \) is the equivalent ratio for the baseline (0 h) sample.

Monitoring of the m+3 and m+2 peaks permitted greater loading of the GC/MS and enhanced the ability to detect low enrichments with good precision, as we do for leucine enrichment in apoB (21). Standards with enrichments of 0.00–1.00 mpe were included at the beginning and end of each batch of samples and used to correct the calculated mpe values with the calculated recovery rate of the standards. Care was taken to ensure similar total ion counts in the standards and all samples.

In three subjects the m/z 148, m/z 147, m/z 146 and m/z 145 peaks was measured to compare the glycerol enrichment by assessing the d5-glycerol tracer as an entire moiety in GC/MS analysis and by the technique described above.

Model Development— The measurements of enrichment of free leucine in plasma, VLDL\(_1\) and VLDL\(_2\), enrichment of glycerol in VLDL\(_1\) and VLDL\(_2\), the pool sizes of leucine and glycerol (\(i.e.,\) derived from apoB and TG) in VLDL\(_1\) and VLDL\(_2\), and the known injected amounts of labeled leucine and glycerol were used to determine kinetic parameters using the modeling software SAAMII (SAAM Institute, Seattle, WA) and Matlab. By simultaneously model apoB and TG kinetics it was possible to
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determine the TG:apoB ratio of newly produced VLDL₁ and VLDL₂ particles and in detail follow the transfer and removal of lipids.

The data were analyzed with two linear compartmental models. The proposed apoB/TG model can be envisioned as a two-layer model, connected at certain points and is based on the apoB model originally described by Packard et al. (25) which has been used in several studies (9,10,25-29). Basically, the model consists of four parts; plasma leucine, plasma glycerol, the assembly of lipoprotein and lipoprotein plasma kinetics.

**Modeling of plasma leucine**— Free plasma leucine was modeled as a four-compartment catenary system (Fig. 1). Compartment 1 is the plasma compartment, where the leucine is injected. Compartments 3 and 4 are protein pools, which give a slow release of leucine. Compartment 2 is an intracellular compartment from which the leucine is transferred into the liver’s apoB synthetic machinery. The transfer coefficients between compartments 1 and 2 are equal, giving equilibrium. To further decrease the number of unknowns, \( k_{3,4} \) is set at 0.1\( k_{4,3} \) (i.e., the transfer from compartment 4 to 3 is one tenth of the transfer from compartment 3 to 4) (26). Other approaches such as a forcing function, determined from measurements could also be used.

**Modeling of plasma glycerol**— TG assembly was modeled with a modified variant of a model described by Zech et al. (4). The plasma compartment (i.e., compartment 13) is connected to an extrahepatic pool (compartment 12). The fractional transfer coefficients are fixed by the population means as described by Zech et al. (4): \( k_{12,13} = 12 \), \( k_{13,12} = 5 \), and \( k_{0,13} = 19 \) [h⁻¹] (Fig. 1). This restriction could be relaxed by measurements of enrichment of free glycerol in plasma and to either choose parameters to fit the data or to use a forcing function determined by the data. To justify the approach of population means we have made measurements of plasma glycerol and compared the kinetic parameters determined by the two models.

**Modeling of lipoprotein assembly**— The TG conversion (compartment 14) has influx from compartment 13, and a slow path for conversion is implemented as compartment 21 interchanging materials with compartment 14. In comparison to the model by Zech et al. (4) the slow pathway was modeled by compartment 21 instead of a compartment with influx from compartment 13 and outfluxes into compartments 5 and 8. The reason for choosing the current model is the reduced complexity of the
model. Both models allow for a slow production pathway (most noticeable after 8 h), but the current implementation does not allow for a small amount of material to rapidly pass through the slow pathway.

In theory, information as to the extent of tracer recycling in the liver through compartment 21 could be obtained by following VLDL kinetics over a prolonged period, e.g., 24 h. In the latter stages the input of tracer into VLDL would be dominated by internally recycled material via compartment 21 (i.e., material stored in compartment 21 and released back to compartment 14). Over the 8 h of the current experiments there is limited ability to define this tail of the VLDL curve with precision (4,30).

The synthesis of apoB and lipoproteins was modeled by two delays, a seven-compartment delay initially set to 0.5 h for apoB and a five-compartment delay initially set to 0.3 h for TG (Fig. 1).

**Modeling of VLDL1 and VLDL2 kinetics**— In the underlying apoB model (Fig. 1), a particle is thought of moving downwards (i.e., to a higher density lipoprotein) as it moves from one compartment to another. The TG:apoB decreases as the density increases. Hence, for an apoB particle to move downward in the model, its carrier lipoprotein must lose TG.

Focusing on the apoB model (Figure 1), the hydrolysis chain is modeled by a four-compartment chain (compartments 5, 6, 8, and 10) and two slowly decaying compartments (7 and 9). VLDL1 consists of compartments 5, 6, and 7, and VLDL2 consists of compartments 8, 10, and 9. Direct removal of apoB (and hence whole particles) is allowed from compartments 6, 7, 9, and 10. Particles in compartment 10 can be removed both by direct removal and by transfer to IDL. However, it is not possible to separate these without sampling IDL apoB enrichments and measuring the pool size.

VLDL-TG kinetics is often modeled by a single compartment (i.e., having monoexponential decay), but our goal was to use the same model used for apoB. This made it possible to extract quantities such as the TG:apoB ratio of newly produced particles. Using different models for apoB and TG would have made it impossible to compare the transfer rates of apoB and TG.

Mathematically, a compartment is defined as an amount of material with homogeneous kinetics. Therefore, all particles in an apoB compartment should be thought of as having similar kinetics and their average TG:apoB ratio is the ratio of the pools. However, there are variations of composition and size within VLDL subfractions, and the true distribution of lipoprotein particles is continuous. Thus, a small
VLDL₁ particle might be smaller than a large VLDL₂ particle, and consequently the TG:apoB ratio of some VLDL₁ particles may be lower than that of some VLDL₂.

The TG model shares the structure of the apoB model, where each apoB compartment (5, 6 etc) have a corresponding TG compartment number 15, 16 etc.

There are several ways to connect the TG model to the apoB model, here we present an intuitive approach were the TG is removed in the transition between two compartments. We denote the TG:apoB ratio in a compartment by $A_i$, and therefore the TG mass in a compartment is $Q(i+10)=A_i \cdot Q_i$. Furthermore, the fraction of the TG that is removed during the transition is denoted $(1-f_{j,i})$. More precisely the TG:apoB ratio of a particle that leaves compartment $i$ is $A_i$, the amount of TG per apoB that is removed from that particle is $(1-f_{j,i}) A_i$ and the TG:apoB ratio of the particles when it enters the destination compartment, $j$, is $f_{j,i} A_i$. Therefore, the fractional transfer coefficients for the TG compartments are defined by

$$k_{j,10,i+10} = f_{j,i} k_{j,i}, \quad k_{0,10+10} = k_{0,10} + \sum_{j=5}^{10} (1-f_{j,i}) k_{j,i}, \quad i, j = 5, ..., 10.$$  

The steady state assumption gives that $A_5$ equals the ratio of the TG and apoB fluxes into compartments 16 and 6. Furthermore $A_6=A_5 \cdot f_{6,5}, A_7=A_5 \cdot f_{7,5}, A_8=A_6 \cdot f_{8,6}$ etc. VLDL₂ particles synthesized in the liver and derived from VLDL₁ should have the same TG:apoB ratio. This is assured by defining the fraction of TG going into VLDL₁, $d_{15}$, as

$$d_{15} = \frac{d_5}{d_5 + f_{6,5} f_{6,5} (1-d_5)}$$  

Where $d_5$ is the fraction of apoB going into VLDL₁. The unknown parameters in the model are the apoB transfer coefficients and the $f_{j,i}$ 's.

An alternative model that gives comparable results (production and catabolic rates), has been described in detail by Adiels (31).

In the model, direct removals from compartments 6 and 16 and 10 and 20 were initially set to zero, and were allowed to be greater than zero only if no satisfactory fit could be achieved. The value for direct removal was then defined as the lowest value to produce a goodness of fit within 1% of maximal goodness of fit (achieved with no limit of direct removal).
The parameters determined in the model are the parameters in the apoB model, i.e. the fractional transfer coefficients between the compartments 1 to 10, with the constraints \( k_{3,4} = 0.1 k_{3,3} \) and \( k_{1,2} = k_{2,1} \) as described above and the delay time as well as the fractions of apoB going to VLDL₁ and VLDL₂ (\( d₁ \) and \( d₈ = 1 - d₃ \)). For the TG the fractional transfer coefficients corresponding to the liver kinetics, \( k_{14,12}, k_{14,21} \) and \( k_{21,14} \), was determined as well the transfer from compartment 14 to the delay compartment, the delay time and the fractions of TG being transferred between compartments, i.e. the \( f_{i,j} \)'s.

**Statistical analysis** — Bivariate correlations were determined by linear regression. Before regression was applied, the data were transformed to achieve a linear relationship. Statistical analysis was performed using Microsoft™ Excel.

**Calculated parameters** — From the calculated solution to the model, we calculated the fractional catabolic rate (FCR) (i.e., total fractional loss of apoB/TG from VLDL₁ and from VLDL₂), the fractional direct catabolic rate (FDCR) (i.e., fractional loss of apoB/TG from VLDL₁ due to direct catabolism and hydrolyzation), and the fractional transfer rate (FTR) (i.e., fractional transfer of apoB/TG from VLDL₁ to VLDL₂). The mean residence time (MRT) for a particle in VLDL₁ and VLDL₂ was calculated as the reciprocal of the corresponding FCR, and the production of apoB and TG were calculated as mg/day/kg body w. The model does not allow for discriminating transfer of VLDL₂ to IDL from direct removal, since IDL is not included in the model.

**List of equations** — The flow of material from a compartment, \( k \), to another compartment, \( l \), or the environment \( l=0 \), is defined as:

\[
\begin{align*}
FTR_{\text{apoB}} &= \frac{\text{FLUX}(8,6)}{Q_8 + Q_6 + Q_7}, \\
FCR_{\text{apoB}}^{\text{VLDL}_1} &= \frac{\text{FLUX}(0,6) + \text{FLUX}(8,6) + \text{FLUX}(0,7)}{Q_1 + Q_6 + Q_7}, \\
FDCR_{\text{apoB}}^{\text{VLDL}_1} &= \frac{\text{FLUX}(0,6) + \text{FLUX}(0,7)}{Q_1 + Q_6 + Q_7}, \\
FCR_{\text{apoB}}^{\text{VLDL}_2} &= \frac{\text{FLUX}(0,9) + \text{FLUX}(0,10)}{Q_4 + Q_6 + Q_{10}}, \\
FTR_{\text{TG}} &= \frac{\text{FLUX}(18,16)}{Q_3 + Q_6 + Q_7}, \\
FCR_{\text{TG}}^{\text{VLDL}_1} &= \frac{\text{FLUX}(0,15) + \text{FLUX}(0,16) + \text{FLUX}(18,16) + \text{FLUX}(0,17)}{Q_3 + Q_6 + Q_7}, \\
FDCR_{\text{TG}}^{\text{VLDL}_1} &= \frac{\text{FLUX}(0,15) + \text{FLUX}(0,16) + \text{FLUX}(0,17)}{Q_3 + Q_6 + Q_7}, \\
FCR_{\text{TG}}^{\text{VLDL}_2} &= \frac{\text{FLUX}(0,18) + \text{FLUX}(0,19) + \text{FLUX}(0,20)}{Q_{13} + Q_{16} + Q_{20}}.
\end{align*}
\]
RESULTS

Catabolic and transfer rates of VLDL subclasses. The 17 subjects (all men) had a mean age of 49 ± 9 years, a mean body mass index of 26.4 ± 2.4 kg/m², and lipid values representative of a Western population (Table 1). The multicompartmental model (Figure 1) was developed and used to determine the kinetics of apoB and TG in VLDL₁ and VLDL₂ in all subjects, and the catabolic and transfer rates for both VLDL₁ and VLDL₂ were calculated (Table 1). Typical enrichment curves and fit to the model are shown in Figure 2.

TG production was 107-347 mg/day/kg in VLDL₁ and 9.5-51.8 mg/day/kg in VLDL₂ (Table 2). These values correspond to 8-30 g/day and 0.7-4.6 g/day, respectively. TG was removed from VLDL₁ by three pathways: particles transferred from VLDL₁ to VLDL₂ (characterized by the VLDL₁ FTR), particles removed by direct catabolism (i.e., VLDL₁ particle uptake by cells), and TG removal by hydrolysis. The latter two are combined into the FDCR.

Parameter free analysis. Analysis of the enrichment curves (Figure 2) shows that the enrichment rapidly increases which indicates that the influx is much greater than the outflux. In the peak area either the influx starts to decrease, or the outflux balances the influx. In the rapid decay interval of the curve the outflux dominates the influx. After the peak of VLDL₁, labeled material enters VLDL₂ both directly from the liver and from VLDL₁.

In the initial part of the enrichment curve all enriched material that has been secreted is within the pool. Let E be the total amount of enriched material secreted and a the fraction that is secreted as VLDL₁. Hence, the fraction secreted as VLDL₂ is (1-a). We denote the VLDL₁ pool P₁ and the VLDL₂ pool P₂, and the enrichment of VLDL₁ E₁ and enrichment of VLDL₂ E₂. Thus, E₁=E*a/P₁ and E₂=E*(1-a)*P₂ and hence E₁/E₂=P₂*a/(P₁*(1-a)) or a = 1-P₂/(P₂+P₁*E₁/E₂). By approximating the initial slope by a straight line we estimated E₁ and E₂, and using the pool size measurements averaged over the 3 time points we calculated the fraction of particles and the fraction of TG going into VLDL₁ and VLDL₂ within a few percent (data not shown).

TG kinetics approximated by apoB kinetics. To further predict the TG model we have used the calculated apoB kinetics to approximate the TG FDCR. The FDCR for TG is composed of direct removal of whole particles and removal of lipids due to hydrolysis. The amount of TG removed due to direct
removal of particles can be estimated by the FDCR(a apoB) times the average TG:apoB ratio. The loss of TG due to lipolysis is the FTR(a apoB) times the difference in size of a VLDL\textsubscript{1} particle and a VLDL\textsubscript{2} particle. There is also an unknown term of the amount of TG that has to be removed to form an average size VLDL\textsubscript{1} particle. Hence, a low estimate of the TG FDCR is

\[
FDCR_{TG} = \frac{FTR_{apoB} \times R_1 + FTR_{apoB} \times (R_1 - R_{1/2})}{R_1}
\]

Using \(R_{1/2} = (R_1 + R_2)/2\) we get estimates of FDCR which are in average 30\% lower than the model derived FDCR. Using average values from the subjects in this study (Table 2) we calculated the FDCR to be 8.2, which should be compared to the average of the model FDCRs which was 11.5.

**Impact of population means for plasma glycerol.** In three subjects, the plasma glycerol enrichment were measured and included in the model and the fractional transfer coefficients between and from compartments 13 and 12 were allowed to vary. These subjects were also modeled with the usual model. Even if the individual transfer coefficients varied between the two models, the derived kinetic parameters, such as productions and the FCR, was within 15\%, suggesting that the use of fixed transfer coefficients gives reasonable results for normal subjects.

**Tracer recycling.** To understand the impact that intra hepatic glycerol recycling between compartments 14 and 21 has on the final VLDL TG kinetic parameters determined in the model, we undertook a sensitivity analysis in which \(k(21,14)\) or \(k(14,21)\) were varied by 100\% from the optimal value and the model was allowed to fit the observed data. It was found that variation in these parameters across this range altered the final VLDL TG production by less than 20\% (VLDL\textsubscript{1} and VLDL\textsubscript{2} production 5.5±5.9\% and 5±4.6\%, respectively) and VLDL clearance by less than 25\% (VLDL\textsubscript{1} FCR 5.4±5.4\%, VLDL\textsubscript{1} FDCR 6.3±6.8\%, and VLDL\textsubscript{2} FCR 6.8±7.7\%). The largest relative change was when lowering \(k(21,14)\) by 50\%, with smaller changes the relative difference was much less. Thus, the model is practically insensitive to hepatic fluxes of glycerol and TG.

**Impact of deuterium atom loss for determination of glycerol enrichment in TG.** It is known that glycerol during metabolic interconversions in the liver can lose H (deuterium atoms). In theory this will result in a reduced apparent enrichment, *i.e.* the \(m+2/m0\) value will vary to some degree depending on the extent to which there has been loss of one deuterium from the fragment which has been detected as
m+3. Therefore, we compared the enrichment curves generated by analysis of the m+3 and m+2 ion mass fragments and by the mass ratios of m+3 (148), m+2 (147), m+1 (146) and m0 (145) in three subjects (Fig. 3). The result showed that the atom percent excess in VLDL1 averaged for three subjects were similar when using a fixed m2/m0 measured at baseline or when using m2/m0 calculated at each time point (Fig. 3). This result shows that the theoretical reduced apparent enrichment made no difference to the kinetic modeling.

**Correlations of plasma TG to calculated values.** Total plasma TG did not correlate significantly with the production rates of TG or apoB in total VLDL or in its subclasses (data not shown). However, total plasma TG did show a negative relationship to VLDL1 FCR in both apoB (p=0.001) and TG (p<0.01) and to VLDL2 FCR (apoB p<0.01; TG, p<0.05).

**Correlations of VLDL1 and VLDL2 concentrations.** Analysis showed significant correlation between VLDL1 apoB concentration and VLDL1 apoB production (p<0.05) and a significant negative correlation between VLDL1 apoB concentration and VLDL1 apoB FCR (p<0.001) (Fig. 4). The VLDL2 apoB concentration did correlate to VLDL2 apoB direct production (p<0.05) and correlated to transfer from VLDL1 (p<0.01) and it showed a negative correlation to VLDL2 FCR (p<0.05). The VLDL1 TG concentration showed a positive correlation to VLDL1 TG production (p<0.05) and a negative correlation to VLDL1 TG FCR (p<0.001) (Fig. 4). Removal of TG from VLDL1 includes the FTR and the FDCR and the FDCR showed a significant inverse correlation with the VLDL1 TG concentration (p<0.001). The FDCR for VLDL1 TG includes both direct removal of TG by catabolism and removal by hydrolysis.

The VLDL2 TG concentration did correlate with VLDL2 TG direct production (p<0.05) and with TG transferred from VLDL1 (p<0.01). There was a trend toward a negative correlation between the VLDL2 TG pool and VLDL2 TG FCR but it was not significant (Fig. 4).

Analysis of the relation between TG and apoB concentrations in circulating VLDL1 and VLDL2 showed a highly significant correlation for both VLDL1 and VLDL2 (Fig. 4). Hence, the average TG:apoB ratio in VLDL1 and VLDL2 varied little between individuals (27.2 ± 3.6 in VLDL1 and 6.1 ± 1.0 in VLDL2) (Table 2).

**Correlations of VLDL1 and VLDL2 production.** The sizes of the secreted VLDL1 or VLDL2 particles from the liver can be estimated from the ratios of TG and apoB in the newly secreted lipoproteins. The
newly secreted VLDL₁ particles were less variable in ratios (i.e. size) than newly secreted VLDL₂ particles; (33.4 ± 8.4) versus (14.3 ± 4.0) (Table 2). There was a strong correlation between VLDL₁ TG and VLDL₁ apoB production (p<0.005). Likewise there was a significant correlation for VLDL₂ direct apoB and TG production (p<0.005) (Fig. 4). The analysis was performed using a linear correlation instead of a log-log correlation because the latter did not give a linear relation of the two quantities, despite almost the same coefficient (r) and significance (P).

*Delay times.* VLDL assembly has been proposed to occur in at least two steps, and it is envisaged that a precursor particle containing apoB₁₀₀ and a small complement of lipid coalesces with a large, apoB-free lipid droplet to form a TG-rich particle (12,13,32). However, support for this concept is based on *in vitro* data only. Therefore, we analyzed the delay times for apoB and TG. The $^2$H₅-glycerol was rapidly incorporated into triacylglycerol and entered the blood system as VLDL TG after 21 ± 3.6 min (mean ± SD). In contrast, apoB₁₀₀ labeled with $^3$H₃-leucine did not appear in the circulation as VLDL apoB until after 33 ± 6 min. Thus, the difference was 12 ± 5.4 min.
DISCUSSION

In this study, we developed a tracer model for apoB100 and TG kinetics in VLDL subclasses and tested it in 17 subjects with lipid values representative of a Western population. We chose to model apoB and TG kinetics simultaneously in an arrangement in which all apoB compartments have corresponding TG compartments, and to couple these at certain points in the model. This strategy made it possible to model both the apoB and TG VLDL₁ and VLDL₂ kinetics and to follow both direct catabolism and transfer of the lipoproteins particles. Before arriving at the current model, we tested several different approaches and alterations. For example, we found it necessary to include two compartments for the hydrolysis chain in both the VLDL₁ and the VLDL₂ submodels. Chains with three or more compartments yielded no improvement, and a single compartment did not fit the data. We also found that it was not necessary to allow the lipoprotein particles to enter the hydrolysis chain in compartments 6 and 10, since this resulted in zero flux into these compartments. In designing the model, we decided to have compartments with particles of uniform size (i.e., uniform TG: apoB ratio) only, since the mass of TG being transferred corresponds to the size of the particles times the number of particles. This limits the model to four distinct particles sizes, apart from the particles in compartments 7 and 9. However, including more compartments, and therefore more possible sizes, did not improve the fit.

We also compared our results with earlier studies. In a recent comprehensive review, Marsh et al. summarized the VLDL FCR from 29 studies, the VLDL production from 27 studies, and the VLDL₁ and VLDL₂ FCRs and production values from eight studies (33). The total VLDL, VLDL₁ and VLDL₂ production, and catabolic rates of the calculated apoB values corresponded well with the values calculated with our multicompartment model. Furthermore, the total TG production corresponded well with the 5–46 g/day for total VLDL TG calculated by Carpentier et al. using a monoexponential model (15). The total VLDL TG FCR varied from 0.22 to 1.2 pools/h, which also corresponded well with the results reported by Carpentier et al. (15). Thus, estimated values extracted from the model compared well with calculated values and results from earlier studies.

Several important findings were gained with the model. Analysis of the correlations showed that plasma TG was determined by the VLDL₁ and VLDL₂ apoB and TG FCR. In fact, both the VLDL₁ and VLDL₂ concentrations are likely determined by their respective FCR, in these subjects. Looking at the actual curves for the relationship between the VLDL₁ and VLDL₂ apoB and TG concentrations and their
respective FCR reveals a curve-linear relationship. When omitting the two subjects with the highest apoB and TG pools only the FCR were significantly correlated to the pool sizes. This indicates that in healthy subjects with low pool sizes the fasting concentrations are determined by the lipolysis rather than the production rate. However, extending the range of plasma TG and VLDL apoB and TG pool sizes might reveal correlation to production rates. This finding is in agreement with studies by Packard et al., who originally showed this for apoB VLDL$_1$ (26).

The model showed a linear correlation between the VLDL$_1$ TG and apoB production. Furthermore, the composition of the VLDL$_1$ and VLDL$_2$ particles (TG:apoB ratio) in plasma suggests that a high level of total plasma TG in achieved mainly by an increased VLDL$_1$ TG pool, but this is the effect of increased number of particles rather than increased particle size. Overall, the contributions of VLDL$_2$ TG pools to the total plasma TG levels were less than that of the VLDL$_1$ TG pools. We speculate that the VLDL$_2$ secretion reflects baseline production of TG, whereas the VLDL$_1$ secretion is modulated by dynamic variations in substrate fluxes. This is consistent with studies showing that insulin does not affect the direct production of VLDL$_2$ apoB or the lipid composition of the VLDL$_2$ particles (9).

The model also revealed a significant difference in the delay times of glycerol and leucine (i.e., VLDL TG and VLDL apoB). After incorporation of glycerol into TG, the completed lipoprotein enters the blood system within 21 minutes. In contrast, the delay time for apoB molecules is 33 minutes. These observations are consistent with a sequential assembly model of VLDL and likely provide the first evidence in man that TG can be added to a primordial apoB-containing particle in the liver (12,32). The data did not show a difference in delay time between VLDL$_1$ and VLDL$_2$. However, such studies need more frequent early sampling times than those used in this study.

In vivo, VLDL$_2$ particles are generated by direct synthesis in the liver or by intravascular lipolysis of VLDL$_1$ particles (10,26). In our model, however, VLDL$_2$ particles had the same kinetics regardless of their origin. This is a tentative caveat because differences in the apolipoprotein or lipid composition of VLDL$_2$ particles from these two sources could affect their kinetics. However, we avoided separating the pathways for these lipoproteins in the model because this simplification did not cause any problem during the modeling and because separation of the pathways would have complicated the model.

Since the planning of these studies improved techniques have been developed for assessing the d5-glycerol tracer as an entire moiety in GC/MS analysis (3,8). The derivation of VLDL TG kinetics using
the GC/MS method used in this study could theoretically lead to the potential loss of deuterium from d5-glycerol tracer during the metabolic process in the liver. However, we found experimentally that this did not invalidate the approach we adopted, but it is possible that higher TG turnover rates would have been obtained by the technique developed by Patterson and coworkers (3,8). For the glycerol model we used population means. We compared the results using population means and the actual plasma glycerol curves in three subjects with the plasma TG of range 1.23-2.77 mmol/L. The derived results (i.e., productions and catabolic rates), were similar in the two models. However, if subjects with severe dyslipidemia are analyzed the populations means for these subjects must be verified or the plasma glycerol enrichments be used. The current data does not allow verifying the actual hepatic TG model. New experiments and emerging knowledge including the quantification of liver fat in man may allow this in future studies.

In summary, we developed and tested a multicompartment model that allows the kinetic parameters of TG and apoB100 in VLDL1 and VLDL2 to be simultaneously determined after a bolus of $^2\text{H}_3$-leucine and $^2\text{H}_5$-glycerol. We suspect that the model will be useful for understanding how the lipid metabolism is disrupted in patients with dyslipidemias.

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FIGURE LEGENDS

Fig. 1. Schematic model of the two-layer compartmental model. The model includes separate modules for leucine and glycerol. The assembly of lipoprotein is modeled by separate delays for apoB and TG. The plasma kinetics is modeled by a four-compartment hydrolysis chain, where the apoB and TG kinetics are coupled at the transfer between compartments. The free leucine plasma kinetics is modeled by two pools (3 and 4) and a plasma compartment (1), which interchange materials with an intracellular compartment (2). Compartment 2 feeds the apoB synthetic machinery. For glycerol, the plasma compartment (13) is connected to a pooling compartment (12) and feeds TG synthesis, which consists of a fast pathway (14) and a slow pathway (21). ApoB synthesis and the synthesis of lipoproteins are modeled by two delays initially set to 0.5 h (leucine) and 0.3 h (glycerol). The plasma kinetics of apoB and TG is modeled by a four-compartment hydrolysis chain, consisting of compartments 5, 6, 8, and 10 for apoB. Each apoB compartment, $i$, has an associated TG compartment, $i+10$, denoted as 15, 16, and so forth. Compartments 5/15 and 6/16 are associated with VLDL$_1$, together with a slowly decaying compartment 7/17. Compartments 8/18 and 10/20 together with the slowly decaying compartment 9/19 form the VLDL$_2$ module. Lipolysis of TG is modeled to take place in the transfer between two compartments. When a particle is being removed from the source, $i$, compartment, only a fraction, $f_{j,i}$, of the TG ends up in the destination compartment, $j$. The rest, $1-f_{j,i}$, is removed by the hydrolysis. The fractional transfer coefficients for the TG compartments are defined by:

$$k_{j+10,i+10} = f_{j,i}k_{j,j}, \quad k_{0,j+10} = k_{0,i} + \sum_{j=5}^{10} (1 - f_{j,i})k_{j,j}, \quad i,j = 5,\ldots,10$$

Hence, direct removal from TG compartments consists of both removal of whole particles (solid arrows) and removal of TG (dashed arrows).

Fig. 2. Typical TG and apoB enrichment curves (subject 7). TG VLDL$_1$ (□, dash-dot) and VLDL$_2$ (◇, dot) are scaled so the TG VLDL$_1$ matches the apoB VLDL$_1$ (●, solid). ApoB VLDL$_2$ is (○, dashed). The VLDL$_1$ curves are similar in shape, but the VLDL$_2$ curves show differences in the initial slope and in clearance. The difference in delay times is clearly shown.

Fig. 3. VLDL$_1$ glycerol enrichment curve averaged for three subjects. To calculate isotope enrichments, the ratio between the ion mass fragments m/z 148 and m/z 147 (i.e., m+3/m+2) was multiplied with a
fixed m/z 147: m/z 145 (i.e., m+2/m0) ratio measured at time point 0 (●) or with a variable m+2/m0 measured at each time point (■). The resulting m/z 148: m/z 145 values (i.e., m+3/m+0) are expressed as atom percent excess (APE). Errors bars indicate min and max.

**Fig. 4. Analysis of apoB or TG concentration versus MRT, and TG:apoB ratios.** Plots of measured apoB (upper left) or TG (upper right) concentration [mg/kg bw] versus FCR [days], measured apoB concentration versus TG concentration [mg/kg bw] (lower left), and apoB production versus TG production [mg/day/kg bw] (lower right). The analysis of VLDL₁ (●) and VLDL₂ (○) was performed on 17 subjects. One of these had a higher cholesterol value (7.5 mmol/L) than the remaining subjects; VLDL₁ (■) and VLDL₂ (□), and three subjects had higher BMI (between 29.5 and 30.1 kg/m²) than the remaining subjects; VLDL₁ (●) and VLDL₂ (◇). The correlation between apoB concentration and apoB FCR in VLDL₁ (P < 0.001) and VLDL₂ (P < 0.05). VLDL₁ TG concentration did correlate with VLDL₁ TG FCR (P < 0.001). The relation between TG and apoB concentrations in circulating VLDL₁ and VLDL₂ showed a highly significant correlation for both VLDL₁ and VLDL₂, (P < 0.001 and P < 0.001, respectively). There was a strong correlation between VLDL₁ TG and VLDL₁ apoB production (P < 0.005) and a correlation between VLDL₂ TG and VLDL₂ apoB production (p<0.005).
Table 1 Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>49 ± 9</td>
<td>25–59</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.2 ± 8.7</td>
<td>69.8–97.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 ± 2.4</td>
<td>22.4–30.1</td>
</tr>
<tr>
<td>Alanine transaminase (IU)</td>
<td>26 ± 7</td>
<td>14–39</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>5.9 ± 2.1</td>
<td>2–10</td>
</tr>
<tr>
<td>P-gluc (mg/dl)</td>
<td>107 ± 10</td>
<td>90.5–130</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.54 ± 0.46</td>
<td>0.99–2.59</td>
</tr>
<tr>
<td>Chol (mmol/l)</td>
<td>5.2 ± 1.0</td>
<td>4.0–7.5</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.34 ± 0.23</td>
<td>0.93–1.68</td>
</tr>
<tr>
<td>FFA (μmol/l)</td>
<td>520 ± 98</td>
<td>319–648</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>104 ± 24</td>
<td>66–140</td>
</tr>
</tbody>
</table>
Table 2. Plasma concentrations of VLDL, TG to apoB ratios, and calculated parameters: transfer rate, catabolic rate, and production rate

<table>
<thead>
<tr>
<th></th>
<th>Measured</th>
<th>Calculated</th>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>VLDL₁ TG pool [mg/kg bw]</td>
<td>19.2 ± 11.4</td>
<td>4.7 – 47</td>
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<tr>
<td>VLDL₂ TG pool [mg/kg bw]</td>
<td>7.2 ± 3.6</td>
<td>2.20 – 14.3</td>
</tr>
<tr>
<td>VLDL₁ apoB pool [mg/kg bw]</td>
<td>0.75 ± 0.52</td>
<td>0.16 – 2.11</td>
</tr>
<tr>
<td>VLDL₂ apoB pool [mg/kg bw]</td>
<td>1.23 ± 0.65</td>
<td>0.29 – 2.64</td>
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<tr>
<td>VLDL₁ TG:apoB pool [mg/mg]</td>
<td>27.2 ± 3.6</td>
<td>22.0 – 33.6</td>
</tr>
<tr>
<td>VLDL₂ TG:apoB pool [mg/mg]</td>
<td>6.08 ± 1.00</td>
<td>4.27 – 7.76</td>
</tr>
<tr>
<td>VLDL₁ apoB prod [mg/mg]</td>
<td>33.4 ± 8.4</td>
<td>19.7 – 49.4</td>
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<tr>
<td>VLDL₂ apoB prod [mg/mg]</td>
<td>14.3 ± 4.0</td>
<td>6.9 – 21.3</td>
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<tr>
<td>VLDL₁ TG FCR [pools/day]</td>
<td>15.2 ± 8.9</td>
<td>5.7 – 35.5</td>
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<td>VLDL₁ TG FDCR [pools/day]</td>
<td>11.5 ± 8.6</td>
<td>4.2 – 33.0</td>
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<tr>
<td>VLDL₁ TG FTR [pools/day]</td>
<td>3.73 ± 1.84</td>
<td>1.53 – 6.82</td>
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<td>VLDL₂ TG FCR [pools/day]</td>
<td>14.0 ± 7.8</td>
<td>5.8 – 39.4</td>
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<td>VLDL₁ TG production [mg/day/kg bw]</td>
<td>218 ± 76</td>
<td>107 – 347</td>
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<tr>
<td>VLDL₂ TG production [mg/day/kg bw]</td>
<td>245 ± 79</td>
<td>138 – 387</td>
</tr>
<tr>
<td>VLDL₁ to VLDL₂ TG transfer [mg/day/kg bw]</td>
<td>61.2 ± 36.8</td>
<td>20.9 – 146</td>
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<tr>
<td>VLDL₂ TG direct production [mg/day/kg bw]</td>
<td>27.2 ± 11.6</td>
<td>9.5 – 52</td>
</tr>
<tr>
<td>VLDL₁ apoB FCR [pools/day]</td>
<td>12.7 ± 7.5</td>
<td>4.31 – 31.0</td>
</tr>
<tr>
<td>VLDL₁ apoB FDCR [pools/day]</td>
<td>5.32 ± 6.28</td>
<td>0 – 23</td>
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<tr>
<td>VLDL₁ apoB FTR [pools/day]</td>
<td>7.34 ± 3.44</td>
<td>2.08 – 13.5</td>
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<tr>
<td>VLDL₂ apoB FCR [pools/day]</td>
<td>5.95 ± 2.74</td>
<td>2.12 – 12.3</td>
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<tr>
<td>VLDL apoB FCR [pools/day]</td>
<td>5.64 ± 2.93</td>
<td>2.21 – 11.2</td>
</tr>
<tr>
<td>VLDL₁ apoB production [mg/day/kg bw]</td>
<td>6.9 ± 2.9</td>
<td>2.88 – 12.5</td>
</tr>
<tr>
<td>VLDL₁ to VLDL₂ apoB transfer [mg/day/kg bw]</td>
<td>4.36 ± 2.44</td>
<td>1.79 – 10.0</td>
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<tr>
<td>VLDL₂ apoB direct production [mg/day/kg bw]</td>
<td>1.9 ± 0.6</td>
<td>1.0 – 3.0</td>
</tr>
<tr>
<td>VLDL apoB production [mg/day/kg bw]</td>
<td>8.9 ± 3.4</td>
<td>4.1 – 15.1</td>
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</table>
APPENDIX

$Q_i$ denotes the tracee mass in compartment $i$. The VLDL$_1$ apoB compartments are 5, 6, 7 and the corresponding VLDL$_1$ TG compartments are 15, 16, 17. The VLDL$_2$ apoB compartments are 8, 9, 10 and the corresponding VLDL$_2$ TG compartments are 18, 19, 20. $L_{apoB}$ is the total amount of apoB synthesized in the liver and $L_{TG}$ the total amount of TG synthesized in the liver. The fraction of apoB going to VLDL$_1$ is denoted $d_5$ and the fraction going to VLDL$_2$ is denoted $d_8=(1-d_5)$. Analogously, the corresponding fractions for TG are denoted $d_{15}$ and $d_{18}$. We get the following equations for compartments 5 and 6

\[
\frac{dQ_5}{dt} = d_5 L_{apoB} - (k_6,5 + k_7,5)Q_5,
\]

\[
\frac{dQ_6}{dt} = k_6,5 Q_5 - (k_8,6 + k_9,6)Q_6,
\]

Equations (1) and (2) describe the apoB kinetics. Multiplying (1) by $A_5$ and (2) by $A_6$. Assuming steady-state we have $A_5$ and $A_6$ constant, and

\[
\frac{dQ_{15}}{dt} = d_5 A_5 L_{apoB} - (k_6,5 + k_7,5)Q_{15},
\]

\[
\frac{dQ_{16}}{dt} = k_6,5 A_6 Q_{15} - (k_8,6 + k_9,6)Q_{16},
\]

Where we have used $A_5 Q_5 = Q_{15}$ and $A_6 Q_6 = Q_{16}$. Defining $A_5$ to be the ratio of the fluxes of TG and apoB going into compartment 5, $A_5 = (d_{15} L_{TG})/(d_5 L_{apoB})$. Furthermore, letting $A_6 = A_5 f_{6,5}$ etc. and rewriting the equations we get

\[
\frac{dQ_{15}}{dt} = d_{15} L_{TG} - (f_{6,5}k_{6,5} + f_{7,5}k_{7,5} + (1 - f_{6,5})k_{6,5} + (1 - f_{7,5})k_{7,5})Q_{15},
\]

\[
\frac{dQ_{16}}{dt} = k_{6,5}f_{6,5}Q_{15} - ((1 - f_{8,6})k_{8,6} + f_{8,6}k_{8,6} + k_{9,6})Q_{16},
\]

\[
k_{16,15} = k_{6,5}f_{6,5}, \quad k_{0,15} = (1 - f_{6,5})k_{6,5} + (1 - f_{7,5})k_{7,5},
\]

Let $k_{17,15} = k_{7,5}f_{7,5}$, $k_{0,17} = k_{0,7}$

\[
k_{18,16} = k_{8,6}f_{8,6}, \quad k_{0,16} = (1 - f_{8,6})k_{8,6} + k_{0,6}
\]

\[
\frac{dQ_{15}}{dt} = d_{15} L_{TG} - (k_{16,15} + k_{0,15})Q_{15},
\]

\[
\frac{dQ_{16}}{dt} = k_{16,15}Q_{16} - (k_{18,16} + k_{0,16})Q_{16},
\]

When a particle is transferred from a compartment to another compartment, only a fraction of the TG reaches the destination compartment. The rest is removed by hydrolysis. However, the source
compartment detects a loss of the whole particles. Here $f_{6,5}$ denotes the fraction of the TG that is kept during the transfer. The general definition for the fractional transfer coefficients in the TG model is

$$k_{j+10, i+10} = f_{j, j} k_{j, j}, \quad k_{0, i+10} = k_{0, j} + \sum_{j=5}^{10} (1-f_{j, j}) k_{j, j}, \quad i, j = 5, \ldots, 10$$

These definitions of fractional transfer coefficients give a system of equations for the transport of TG that is similar to the transport of apoB. Both systems of equations fulfill the requirements for compartmental systems. The parameters to determine in the model are the $f_{j, i}$'s.

The data available does not allow for separation of pathways for VLDL$_2$ secreted directly from the liver and VLDL$_2$ produced from VLDL$_1$ particles as described (25). Therefore, we constrain the model by defining newly secreted VLDL$_2$ particles and particles transferred from VLDL$_1$ (from compartment 6 to compartment 8) to have the same TG:apoB ratio ($A_8$). Thus, the fraction of TG secreted into VLDL$_1$ and VLDL$_2$ is:

$$A_8 = \frac{(1-d_{15})L_{TG}}{(1-d_5)L_{apoB}} = f_{8,6} f_{6,5} A_5 = f_{8,6} f_{6,5} \frac{d_{15} L_{TG}}{d_5 L_{apoB}}.$$  

Or $d_{15} = \frac{d_5}{d_5 + f_{8,6} f_{6,5} (1-d_5)}$