Development of a novel method to determine very low density lipoprotein kinetics

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Abstract Isotopic tracer methods of determining triglyceride-rich lipoprotein (TRL) kinetics are costly, time-consuming, and labor-intensive. This study aimed to develop a simpler and cost-effective method of obtaining TRL kinetic data, based on the fact that chylomicrons compete with large VLDL (VLDL1; Sf = 60–400) for the same catalytic pathway. Ten healthy subjects [seven men; fasting triglyceride (TG), 44.3–407.6 mg/dl; body mass index, 21–35 kg/m2] were given an intravenous infusion of a chylomicron-like TG emulsion (Intralipid; 0.1 g/kg bolus followed by 0.1 g/kg/h infusion) for 75–120 min to prevent the clearance of VLDL1 by lipoprotein lipase. Multiple blood samples were taken during and after infusion for separation of Intralipid, VLDL1, and VLDL2 by ultracentrifugation. VLDL1-apolipoprotein B (apoB) and TG production rates were calculated from their linear increases in the VLDL1 fraction during the infusion. Intralipid-TG clearance rate was determined from its exponential decay after infusion. The production rates of VLDL1-apoB and VLDL1-TG were (mean ± SEM) 25.4 ± 3.9 and 1,076.7 ± 224.7 mg/h, respectively, and the Intralipid-TG clearance rate was 66.9 ± 11.7 pools/day. Kinetic data obtained from this method agree with values obtained from stable isotope methods and show the expected relationships with indices of body fatness and insulin resistance (all P < 0.05). The protocol is relatively quick, inexpensive, and transferable to nonspecialist laboratories.—Al-Shayji, I. A. R., J. M. R. Gill, J. Cooney, S. Siddiqui, and M. J. Caslake. Development of a novel method to determine very low density lipoprotein kinetics. J. Lipid Res. 2007. 48: 2086–2095.

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A large body of evidence suggests that increased circulating concentrations of triglyceride-rich lipoproteins (TRLs) increase the risk of atherosclerosis (1, 2). This is particularly evident in the postprandial state (3, 4). However, the measurement of a high TRL concentration provides no information regarding the mechanisms responsible for this increase (i.e., increased rate of synthesis and/or reduced rate of catabolism). As it is important to understand the mechanisms responsible for increased TRL concentrations in different metabolic states, both to advance basic scientific understanding and to help guide therapeutic treatments, studies investigating the kinetics of TRL can yield useful data. Such an approach, for example, has revealed that the dyslipidemia associated with insulin resistance and type 2 diabetes is largely attributable to an over-production of heparinically derived large VLDL (VLDL1; Svedberg flotation rate (Sf) = 60–400) (5, 6). These studies usually use precursors labeled with stable or radioactive isotopes to measure the synthesis of lipids and apolipoproteins directly (7–10). Although these techniques yield detailed kinetic data, they are costly, time-consuming, and labor-intensive and require the use of specialized equipment and techniques in research laboratories.

The aim of this study, therefore, was to develop a relatively straightforward method of obtaining TRL kinetic data. The method relies on the fact that chylomicrons compete with VLDL1 particles for the same catalytic pathway [i.e., hydrolysis of their triglyceride (TG) content by the action of LPL]. Previous studies (11, 12) have shown that VLDL1 accumulates in plasma after fat ingestion or intravenous infusion of a lipid emulsion (e.g., Intralipid) as a result of the presence of the newly secreted chylomicrons or chylomicron-like particles, which are the preferred substrate for LPL because of their larger size and TG content (13). Indeed, using stable isotope methods, Björkergen et al. (12) demonstrated that infusion of Intralipid prevents >90% of VLDL1 catabolism. Therefore, we hypothesized that it would be possible to calculate the production rates of VLDL1-TG and VLDL1-apolipoprotein B (apoB) from the rate of their accumulation during an

Abbreviations: apoB, apolipoprotein B; BMI, body mass index; FCR, fractional catabolic rate; FSR, fractional synthetic rate; HOMAIR, homeostasis model assessment insulin resistance; Sf, Svedberg flotation rate; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

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insulin resistance; TG, triglyceride.

**Materials and Methods**

**Subjects**

Ten nonsmoking healthy subjects (seven males and three females) were included in this study after giving written informed consent. All subjects had normal thyroid, liver, and renal function, and none had acute illness, a history of known cardiovascular disease and hypertension, or were under medication known to influence carbohydrate or lipid metabolism. The subjects' characteristics are shown in Table 1. Subjects were requested not to exercise for 3 days before their tests, as this is known to affect TRL metabolism (16). In addition, they were asked to weigh and record their dietary intake for 2 days before the Intralipid test, and this diet was replicated in those subjects who underwent a second Intralipid test. The study protocol was approved by the Research Ethics Committee of the North Glasgow University Hospitals National Health Service Trust.

**Intravenous Intralipid Test**

Each subject reported to the Clinical Investigation Suite in the Department of Vascular Biochemistry at Glasgow Royal Infirmary after an overnight fast of 12 h. Transportation to the hospital was provided for the subjects, when needed, to ensure that they arrived in a rested state. A cannula was introduced into an antecubital vein in both arms, one for administration of Intralipid (purified soybean oil emulsion; Fresenius Kabi, Ltd., Warrington, UK) and the other for blood sampling. The cannulae were kept patent by flushing with nonheparinized saline solution (0.9% NaCl). Ten minutes after cannulation, a first baseline blood sample was obtained. A second baseline blood sample was obtained 10 min later.

The intravenous Intralipid test used was a modification of that described by Björkegren et al. (12). A bolus dose of 20% Intralipid (0.1 g/kg body mass) was injected within 1 min. This was followed immediately by a constant continuous infusion of 10% Intralipid (0.1 g/kg/h). This dose was chosen as Björkegren et al. (12) reported that the rate of increase of VLDL₁-apoB during Intralipid infusion was no greater for a 0.2 g/kg/h infusion dose compared with 0.1 g/kg/h, suggesting that the lower dose was sufficient to saturate LPL and prevent measurable VLDL₁ catabolism. However, we also performed experiments with the 0.2 g/kg/h dose ourselves to confirm that this was the case in our hands (see below). Initially, the infusion period was 120 min; however, during development of the technique, this was subsequently decreased to 75 min after it became clear that a 75 min infusion was long enough to induce a sufficient measurable increase in VLDL₁-TG and VLDL₁-apoB.

Blood samples were obtained at 15 min intervals during the infusion. Further blood samples were drawn at 2.5, 5, 10, 15, 20, 30, 45, 60, and 75 min after infusion. Initially, the postinfusion period was 3.25 h. However, this was subsequently decreased to 75 min when it became clear that this was sufficient to calculate the Intralipid-TG clearance rate using the exponential decay. All samples were obtained directly into potassium EDTA tubes (BD Vacutainer Systems, Plymouth, UK) and placed immediately in ice before centrifuging for 15 min at 3,000 rpm and 4°C.

Aliquots of plasma were frozen immediately at −70°C for subsequent analysis of insulin, NEFA, glucose, TG, total cholesterol, and HDL cholesterol. The remaining plasma was stored overnight at 4°C before separation of Intralipid and lipoproteins.

**Increasing the Intralipid Infusion Rate**

Five subjects [two females and three males; age, 23–47 years (range); body mass index (BMI), 20.8–28.7 kg/m²; fasting TG, 53.2–230.4 mg/dl] underwent a second test using a higher infusion dose (0.2 g/kg/h) of 10% Intralipid with the same 0.1 g/kg bolus dose. This was done to determine whether the Intralipid infusion dose of 0.1 g/kg/h was sufficient to completely prevent measurable lipolysis of VLDL₁ by LPL and, therefore, enable determination of VLDL₁-TG and VLDL₁-apoB production rates from their increases in concentration. If the infusion rate of 0.1 g/kg/h was sufficient to saturate LPL and block lipolysis of VLDL₁, the 0.2 g/kg/h dose would not result in higher calculated production rates of VLDL₁-TG or VLDL₁-apoB compared with the 0.1 g/kg/h dose. The order of testing was randomized. Other than the higher infusion dose, all conditions of the tests were the same.

**Intralipid (S₁ > 400) Separation from Whole Plasma**

Two milliliters of plasma were overlaid with 4 ml of 1.006 g/ml density solution in ultracentrifuge tubes and spun at 10,000 rpm and 4°C for 30 min (17) using a Beckman L8-M Ultracentrifuge and a Beckman 50.4 rotor (Beckman Instruments, Inc.). Intralipid (d < 1.006 g/ml) was removed in the top 2 ml (IL-1) for subsequent measurements of TG using commercially available enzymatic colorimetric kits (Roche Diagnostics GmbH, Mannheim, Germany). TG concentration was also measured in the middle 1.5 ml fraction (IL-2) to verify complete separation of Intralipid. The final Intralipid-TG concentration was calculated as the addition of these two fractions [IL-1 + (IL-2 × 1.5/2)]. In addition, glycerol was measured in these IL-1 and IL-2 fractions using commercially available kits (Randox Laboratories, Ltd.) to determine the amount of free glycerol. The final 0.5 ml of the density solution was discarded, and the remaining 2 ml of Intralipid-free plasma was used for the separation.

**Table 1.** Subject physical and metabolic characteristics (n = 10)

| Characteristic                  | Mean    | Range              |
|--------------------------------|---------|--------------------|
| Age (years)                    | 33.5    | (20.0–55.0)        |
| BMI (kg/m²)                    | 25.9    | (20.8–34.7)        |
| Waist circumference (cm)       | 85.1    | (65.0–113.5)       |
| Waist-hip ratio                | 0.84    | (0.71–1.04)        |
| TGs (mg/dl)                    | 120.72  | (35.44–392.50)     |
| Total cholesterol (mg/dl)      | 160.31  | (110.1–227.74)     |
| HDL cholesterol (mg/dl)        | 50.81   | (28.95–71.22)      |
| LDL cholesterol (mg/dl)        | 85.30   | (47.79–154.40)     |
| Glucose (mg/dl)                | 98.48   | (77.40–144.00)     |
| Insulin (mU/l)                 | 8.92    | (2.78–24.81)       |
| HOMA IR                        | 2.41    | (0.56–8.82)        |
| NEFA (mEq/l)                   | 0.51    | (0.34–0.70)        |

BMI, body mass index; HOMA IR, homeostasis model assessment insulin resistance; TG, triglyceride.
of VLDL$_1$ and VLDL$_2$. The coefficient of variation (CV) for the Intralipid-TG separation was 6.9%.

**VLDL$_1$ and VLDL$_2$ separation**

VLDL$_1$ (S$_r$ = 60–400) and VLDL$_2$ (S$_r$ = 20–60) were isolated from plasma using a modification of the cumulative ultracentrifugation density gradient technique described by Lindgren, Jensen, and Hatch (18). TG concentrations were then measured in the VLDL$_1$ and VLDL$_2$ fractions at all time points using commercially available kits as described previously. ApoB concentrations were also measured directly by immunoturbidimetry using commercially available kits (WAKO Apolipoprotein B-BA; Wako Chemicals GmbH). The CVs for the separation of VLDL$_1$-TG and VLDL$_1$-apoB were 5.0% and 3.4%, respectively, and those for VLDL$_2$-TG and VLDL$_2$-apoB were 5.8% and 1.4%, respectively.

**Fasting plasma analysis**

Plasma glucose, total cholesterol, and HDL cholesterol concentrations in the fasted state and TG and NEFA concentrations at all time points were analyzed using commercially available enzymatic colorimetric kits (glucose hexokinase (Randox Laboratories, Ltd.); total cholesterol and HDL cholesterol (Roche Diagnostics GmbH); free fatty acid (Wako Chemicals USA, Inc.)). ApoB concentrations were also measured directly by immunoturbidimetry using commercially available kits (Mercodia Insulin ELISA).

**Correction for glycerol**

Enzymatic kits for TG analysis measure the glycerol that is hydrolyzed from TG by LPL. As Intralipid contains free glycerol as an excipient, it has been reported that it overestimates the total TG concentrations of Intralipid (19). Therefore, all Intralipid-TG measurements were corrected for free glycerol and are reported as “true” TG concentrations [true TG concentration (mg/dl) = [measured TG (mmol/l) – glycerol (mmol/l)] × 88.6]. Glycerol concentrations were also measured in five subjects in the VLDL$_1$ fraction during infusion and were found to be negligible (influencing VLDL$_2$-TG concentrations by <1%).

**Kinetic data calculations**

The clearance rates of Intralipid-TG and production rates of VLDL$_1$-TG and VLDL$_1$-apoB were calculated as described below using examples from individual subjects.

**Calculating VLDL$_1$-TG and VLDL$_1$-apoB production rates.** The production rates (mg/h) of VLDL$_1$-TG and VLDL$_1$-apoB were calculated from the linear rate of increase in their concentrations (mg/dl) over time (min) multiplied by plasma volume [4% of body mass (20)] in deciliters and then by 60 min. Figure 1A represents the linear increase in TG (mg/dl) in the VLDL$_1$ fraction of subject 3 (female, 55 years, 84.5 kg), with $R^2$ (goodness-of-fit) value of 0.97 and a gradient of 1.0589. Assuming this subject’s plasma volume is 3.38 liters (33.8 dl), the VLDL$_1$-TG production rate would be $(1.0589 \times 33.8 \times 60) \times 2.1474$ mg/h (609.9 mg/kg/day). Similarly, from Fig. 1B, the VLDL$_1$-apoB production rate of the same subject was 50.9 mg/h (14.5 mg/kg/day).

**Calculating VLDL$_1$-TG and VLDL$_1$-apoB fractional synthetic and catabolic rates.** The fractional synthetic rate (FSR) is defined as the rate of incorporation of a precursor into a product per unit of product mass (21), which can be calculated as:

$$\text{FSR} = \frac{\text{initial rate of change in product}}{\text{initial precursor concentration}} \quad (\text{Eq. 1})$$

**Calculating Intralipid-TG clearance rate.** Assuming that all TG clearance is Intralipid-TG clearance, it is possible to determine Intralipid-TG clearance in two ways:

**The Steady-State Method.** The clearance rate of Intralipid-TG can be calculated from the steady-state concentration during infusion using the following equation (14):

$$\text{clearance rate (ml/min)} = \frac{\text{infusion rate (mg/min)}}{\text{steady-state concentration (mg/ml)}} \quad (\text{Eq. 2})$$

In this method, we defined that a steady state was achieved when the final three values of the Intralipid-TG concentrations differed by <13.8% (i.e., two times the CV for the separation of the Intralipid fraction and measurement of the TG; this represents the 95% confidence interval for the measured value). To calculate the clearance rate in pools per day, the values were divided by plasma volume (4% of body mass) and then multiplied by 60 min and 24 h.

**The Exponential Method.** After stopping the intravenous infusion, Intralipid-TG declines exponentially according to first-order kinetics as described by Rössner (15). The Intralipid-TG clearance rate can be calculated from the Intralipid-TG concentrations (mg/dl) after infusion over time (min) curve plotted on a semilog scale. The equation of the fitted line is:

$$y = k e^{-bt} \quad (\text{Eq. 3})$$
TABLE 2. Fasting concentrations and individual lipoprotein kinetic parameters calculated using the Intralipid method (0.1 g/kg/h infusion dose) in the 10 subjects studied

| Subject | VLDL1-TG | VLDL1-ApoB | VLDL2-TG | VLDL2-ApoB |
|---------|----------|------------|----------|------------|
| 1       | 19.9     | 0.8        | 1.6      | 625.0      |
| 2       | 20.8     | 1.1        | 2.9      | 719.9      |
| 3       | 49.4     | 1.8        | 6.8      | 2,147.4    |
| 4       | 10.2     | 0.8        | 0.9      | 642.3      |
| 5       | 13.7     | 1.1        | 2.2      | 711.2      |
| 6       | 226.8    | 10.6       | 7.7      | 2,563.2    |
| 7       | 109.4    | 3.8        | 3.7      | 1,228.1    |
| 8       | 11.5     | 0.3        | 3.0      | 440.0      |
| 9       | 35.7     | 1.0        | 3.0      | 885.5      |
| 10      | 28.6     | 0.5        | 2.9      | 798.2      |

| Production Rates | Intralipid-TG Clearance Rate | FSRb |
|------------------|------------------------------|------|
| mg/h             | mg/kg/day                    | pools/day |
| 1                | 17.1                         | 30.2 |
| 2                | 14.8                         | 25.8 |
| 3                | 11.4                         | 56.9 |
| 4                | 12.0                         | 20.7 |
| 5                | 14.8                         | 4.4  |
| 6                | 16.1                         | 4.4  |
| 7                | 16.1                         | 4.4  |
| 8                | 17.1                         | 4.4  |
| 9                | 18.1                         | 4.4  |
| 10               | 20.7                         | 4.4  |

| Fasting Concentrations | Production Rates | Intralipid-TG Clearance Rate | FSRb |
|------------------------|------------------|------------------------------|------|
| VLDL1-TG               | VLDL1-ApoB       | VLDL2-TG                     | VLDL2-ApoB |
| mg/dl                  | mg/h             | mg/kg/day                    | pools/day |
| 1 5                    | 17.1             | 30.2                         | 25.8 |
| 2 4                    | 14.8             | 20.7                         | 10.3 |
| 3 3                    | 11.4             | 4.4                          | 19.8 |
| 4 2                    | 12.0             | 4.4                          | 13.7 |
| 5 1                    | 14.8             | 4.4                          | 13.7 |
| 6 5                    | 16.1             | 4.4                          | 13.7 |
| 7 4                    | 16.1             | 4.4                          | 13.7 |
| 8 3                    | 17.1             | 4.4                          | 13.7 |
| 9 2                    | 18.1             | 4.4                          | 13.7 |
| 10 1                   | 20.7             | 4.4                          | 13.7 |

ApoB, apolipoprotein B; FCR, fractional catabolic rate; FSR, fractional synthetic rate.

a Subjects 3, 5, and 8 were female subjects.
b As FSR equals FCR under steady-state conditions, the VLDL1-TG and VLDL1-apoB FSR values are equal to the FCR values in the fasted state.

where k is the proportionality constant, t is the time, and b is the exponential decay constant, which in turn is defined as:

\[ b = \frac{\text{clearance rate (ml/min)}}{\text{plasma distribution volume (ml)}} \]  \hspace{1cm} (Eq. 4)

Hence,

\[ \text{clearance rate (ml/min)} = b \times \text{plasma volume (ml)} \]  \hspace{1cm} (Eq. 5)

Intralipid recovery

To assess the recovery of plasma-Intralipid in the Intralipid fraction (Sf > 400), EDTA plasma was spiked with Intralipid to produce an Intralipid-TG concentration in plasma of ~133 and ~354 mg/dl. These reflect approximate Intralipid-TG concentrations at the 0.1 and 0.2 g/kg/h infusion doses. For each Intralipid concentration, samples of spiked plasma were divided into 10 aliquots, and the Intralipid fractions were separated as described above. TG and glycerol concentrations were measured in plasma before and after addition of the Intralipid (to calculate the actual Intralipid-TG concentration) as well as in the separated Intralipid fractions. The Intralipid recovery was calculated as follows:

\[ \% \text{ recovery} = \frac{\text{separated Intralipid-TG}}{\text{actual Intralipid-TG}} \times 100 \]  \hspace{1cm} (Eq. 6)

where actual Intralipid-TG = total TG (plasma with Intralipid) – TG (Intralipid-free plasma).

Statistical analyses

Statistical analyses were performed using MINITAB for Windows (version 13.1; MINITAB, Inc., State College, PA) and STATISTICA (release 6.0; StatSoft, Inc.). Normality was checked for all of the data using the Anderson-Darling test. When data did not approximate a normal distribution, these were log-transformed, specifically TG, glucose, homeostasis model assessment insulin resistance (HOMAIR), production rates of VLDL1-TG (expressed in both mg/h and mg/kg/day) and VLDL1-apoB (expressed in mg/h), Intralipid-TG clearance rate, and VLDL1-apoB FSR required transformation. Time trends were tested using one-way ANOVA with repeated measures. Paired t-tests were used to compare between the Intralipid-TG clearance rates calculated from the steady state and the exponential decay and between the kinetic data obtained from the low and high Intralipid doses. The HOMA was used as a validated surrogate measure of insulin resistance (22). Relationships between HOMAIR, NEFA, BMI, waist circumference, and kinetic parameters were assessed using Pearson product-moment correlations. Significance was accepted at the P < 0.05 level. Data are presented as means ± SEM unless stated otherwise.

RESULTS

Plasma-, Intralipid-, VLDL1-, and VLDL2-TG concentrations during and after infusion

Figure 2A shows the mean plasma-, Intralipid-, VLDL1-, and VLDL2-TG responses in 10 subjects during 75 min Intralipid infusion (0.1 g/h/kg body mass). Plasma-TG concentrations increased to approximately two to three times the fasting value during the infusion. Similarly, mean Intralipid-TG concentrations increased to approximately two to three times the fasting value during the infusion. After stopping the infusion, the plasma- and Intralipid-TG concentrations decreased exponentially (both P < 0.01; Fig. 2B). VLDL1-TG continued to increase for ~20 min before plateauing and subsequently decreasing. In subjects in whom the postinfusion period was extended, VLDL1-TG returned to baseline concentrations within 105–135 min (data not shown). The mean VLDL2-TG concentrations remained unchanged for the 75 min postinfusion observation period.

VLDL1-apoB and VLDL2-apoB concentrations during infusion

The mean apoB concentration in the VLDL1 (Sf = 60–400) fraction increased steadily from fasting levels throughout the infusion and was significantly higher than baseline within 15 min (P < 0.001). On the other hand, mean VLDL2-apoB concentrations declined significantly (P < 0.001).
during 75 min of infusion. Total (VLDL<sub>1</sub> + VLDL<sub>2</sub>) VLDL-apoB concentrations increased slightly but significantly \((P < 0.05)\) during infusion (Fig. 3). There was no significant change in the VLDL<sub>1</sub>-TG/apoB or VLDL<sub>2</sub>-TG/apoB ratio (expressed in mmol/mmol) over the 75 min of infusion \((P = 0.21\) and \(P = 0.16\), respectively).

**Kinetic data**

Table 2 shows the production rates and FSR of VLDL<sub>1</sub>-TG and VLDL<sub>1</sub>-apoB as well as the clearance rates of Intralipid-TG calculated for each subject \((n = 10)\) as described previously. Fasting VLDL<sub>1</sub>-TG and VLDL<sub>1</sub>-apoB and VLDL<sub>2</sub>-apoB concentrations are also presented.

**VLDL<sub>1</sub>-TG and VLDL<sub>1</sub>-apoB production rates.** The mean ± SEM (range) production rates for VLDL<sub>1</sub>-TG and VLDL<sub>1</sub>-apoB were 1,076.7 ± 224.7 (446.0–2,563.2) mg/h and 25.4 ± 3.9 (12.0–50.9) mg/h, respectively. These corresponded to 333.6 ± 49.1 (198.5–609.9) mg/kg/day and 8.1 ± 0.9 (4.4–14.5) mg/kg/day, respectively.

**VLDL<sub>1</sub>-TG and VLDL<sub>1</sub>-apoB FSRs and FCRs.** The mean ± SEM (range) VLDL<sub>1</sub>-TG and VLDL<sub>1</sub>-apoB FSRs, which are equal to the VLDL<sub>1</sub>-TG and VLDL<sub>1</sub>-apoB FCRs in the fasted state \((21)\), were 30.2 ± 5.7 (6.5–57.8) pools/day and 21.1 ± 5.1 (2.2–56.2) pools/day, respectively.

**Intralipid-TG clearance rate.** The Intralipid-TG clearance rates calculated for individual subjects by the two methods described above \((i.e.,\) steady state and exponential) are shown in Table 2. Eight of the 10 subjects reached the defined steady state during infusion. The mean ± SEM Intralipid-TG clearance rates in these eight subjects did not differ significantly between the two calculation methods \((52.4 ± 8.6\) pools/day for steady state versus 55.3 ± 9.2 pools/day for exponential; \(P = 0.45)\), and the values obtained were strongly correlated \((r = 0.96, P < 0.001)\). However, because not all subjects reached a steady state, the Intralipid-TG clearance rates mentioned hereafter will refer to those calculated using the exponential method.

**Effect of increasing the Intralipid infusion rate**

For the five subjects who underwent Intralipid infusion at the low \((0.1\) g/kg/h) and high \((0.2\) g/kg/h) doses, there were no significant differences in the mean VLDL<sub>1</sub>-apoB production rates \((\text{low dose}, 23.8 ± 2.8\) vs. high dose, 22.0 ± 1.9 mg/h; \(P = 0.21)\) or VLDL<sub>1</sub>-TG production rates \((\text{low dose}, 813.8 ± 127.0\) vs. high dose, 960.9 ± 136.8 mg/h; \(P = 0.10)\) between the low and high doses, although there was a tendency for the VLDL<sub>1</sub>-TG production rate to be higher at the high Intralipid dose. However, we had observed that separation of the large amount of Intralipid from plasma at the higher \((0.2\) g/kg/h) dose was technically quite difficult and suspected that the VLDL<sub>1</sub> fraction in some samples may have become slightly contaminated with Intralipid at this dose. This suggestion is supported by the substantially lower recovery of Intralipid in the Intralipid fraction at the higher \((0.2\) g/kg/h) dose than at the lower \((0.1\) g/kg/h) dose. Calculated FSRs for VLDL<sub>1</sub>-TG \((\text{low dose}, 30.1 ± 8.8)\) vs. high dose, 30.4 ± 8.1 pools/day; \(P = 0.94)\) and VLDL<sub>1</sub>-apoB \((\text{low dose}, 25.8 ± 8.7)\) vs. high dose, 33.4 ± 14.6 pools/day; \(P = 0.64)\) did not differ between the two doses, and FSRs for VLDL<sub>1</sub>-TG \((r = 0.88, P = 0.05)\) and VLDL<sub>1</sub>-apoB \((r = 0.95, P = 0.01)\) between the two doses correlated highly with each other and, when plotted, followed the line of equality (Fig. 4).

**Intralipid recovery**

Recovery of the Intralipid-TG in the Intralipid \((S_I > 400)\) fraction was 95 ± 7% \((\text{mean ± SD})\) for the ~133 mg/dl
Interrelationships between VLDL1-TG and VLDL1-apoB production and TG clearance

Intralipid-TG clearance rate and VLDL1-TG and VLDL1-apoB production rates (expressed in mg/h) were significantly interrelated, with the expected negative correlation between Intralipid-TG clearance and VLDL1-TG ($r = -0.67$, $P = 0.04$) and VLDL1-apoB ($r = -0.69$, $P = 0.03$) production rates and a positive correlation between VLDL1-TG and VLDL1-apoB production rates ($r = 0.85$, $P = 0.002$). There was also a very strong relationship between VLDL1-TG FSR (which equals the VLDL1-TG FCR in the fasted state) and Intralipid-TG clearance rate ($r = 0.90$, $P < 0.005$). The positive correlation between VLDL1-TG and VLDL1-apoB production rates remained significant between production rates when values were expressed in mg/kg/day ($r = 0.73$, $P = 0.02$). However, the relationships between VLDL1-TG production rate expressed in mg/kg/day and Intralipid-TG clearance ($r = -0.46$, $P = 0.18$) and between VLDL1-apoB production rate expressed in mg/kg/day and Intralipid-TG clearance ($r = -0.28$, $P = 0.44$) were not statistically significant.

Relationships between kinetic variables and subject characteristics

Figure 5 shows the relationships between the measured kinetic variables and subject characteristics, with VLDL1-TG and VLDL1-apoB production rates expressed in mg/h. VLDL1-TG and VLDL1-apoB production rates correlated strongly and significantly with waist circumference and fasting TG concentration. VLDL1-TG production rate also correlated significantly with HOMA IR. Similarly, Intralipid-TG clearance rate was significantly and inversely correlated with waist circumference, fasting TG concentrations, and HOMA IR. In addition, BMI correlated significantly and positively with VLDL1-TG ($r = 0.83$, $P = 0.003$) and VLDL1-apoB ($r = 0.81$, $P = 0.004$) production rates and inversely with Intralipid-TG clearance rate ($r = -0.60$, $P = 0.07$). Fasting NEFA concentrations were not significantly correlated with any of the kinetic variables. The relationships between VLDL1-TG and VLDL1-apoB production rates expressed in mg/kg/day with BMI, waist circumference, fasting TG concentration, and HOMA IR are shown in Table 3. The correlations between VLDL1-TG production and all of these variables remained strong and statistically significant; however, the correlations between VLDL1-apoB production rate and waist circumference and fasting TG were not statistically significant when the production rates were normalized for body mass.

DISCUSSION

In this study, we have developed a relatively straightforward method of determining TRL kinetics. The method relies on the fact that chylomicrons or chylomicron-like particles, such as Intralipid, compete with hepatically derived large VLDL particles for clearance by a common saturable pathway [i.e., hydrolysis of their TG content by LPL (11, 12)] and that chylomicrons or chylomicron-like particles are the preferred substrate for LPL (13). Thus, the presence of a sufficient concentration of chylomicrons or chylomicron-like particles in the circulation will almost entirely prevent the clearance of VLDL by LPL (12); therefore, the rates of VLDL1-TG and VLDL1-apoB production can be calculated from their rates of increase in concentration. This work builds on the findings of Björkegren et al. (12), who, in studies designed to evaluate the effects of Intralipid infusion on VLDL1 ($S_t = 60–400$) and VLDL2 ($S_t = 20–60$) kinetics, found that individual rates of VLDL1-apoB production calculated from the rate of increase of VLDL1-apoB during infusion were virtually identical to those calculated from the “gold-standard” stable isotope method (see below). The Intralipid method described here enables the determination of the rates of VLDL1-TG (i.e., VLDL1 lipid) and VLDL1-apoB (i.e., VLDL1 particle) production as well as the clearance rates of chylomicron-like particles.

The Intralipid method specifically measures the production rate of large VLDL1 rather than total VLDL (i.e., $S_t = 20–400$). VLDL is a metabolically heterogeneous class of lipoproteins, and it is the larger VLDL1 subclass that competes with chylomicrons/chylomicron-like particles.
for LPL-mediated clearance and would have its clearance blocked by the presence of Intralipid (12). In contrast, catabolism of the smaller VLDL2 subclass would not be blocked completely by Intralipid, as its clearance can occur via the action of hepatic lipase as well as LPL (23). Indeed, as one source of VLDL2 is from the catabolism of VLDL1 (the other being direct hepatic production) and this process was blocked by Intralipid infusion, mean VLDL2-apoB concentrations decreased slightly during the infusion, although individual responses were more heterogeneous than those observed with VLDL1-apoB, a finding also reported by Björkegren et al. (12). This heterogeneity in individual VLDL2-apoB responses meant that it was not possible to perform any kinetic analyses using the VLDL2 data.

To validate the calculation of VLDL1-apoB and VLDL1-TG production rates using the Intralipid method, it was necessary to consider a number of issues. The first was to determine whether infusing a higher Intralipid dose would influence the calculated VLDL1-apoB and VLDL1-TG production rates. This was necessary to establish whether the proposed Intralipid infusion dose of 0.1 g/kg/h was sufficient to saturate LPL and block the clearance of VLDL1: if the 0.1 g/kg/h dose was sufficient, infusing a higher Intralipid dose should not affect the calculated production rates. In agreement with the findings of Björkegren et al. (12), we found that the calculated VLDL1-apoB production rate was not changed when a higher (0.2 g/kg/h) Intralipid infusion dose was used. Similarly, FSRs, which

![Graphs showing scatterplots with linear regression lines for VLDL1-apoB production rate, VLDL1-TG production rate, and Intralipid-TG clearance rate against subject characteristics.](image-url)
correspond to the FCRs in the fasted state, for VLDL$_1$-apoB and VLDL$_1$-TG did not differ between the two doses (Fig. 4). We did observe a tendency for the calculated VLDL$_1$-TG production rate to be higher with the 0.2 g/kg/h dose, although this was not statistically significant. However, we feel that the slightly higher apparent VLDL$_1$-TG production rate at the high dose was a methodological, rather than a physiological, issue caused by the difficulty in separating Intralipid at the high dose, leading to the potential contamination of VLDL$_1$ fraction with Intralipid. This is supported by the fact that Intralipid recoveries at high Intralipid doses were relatively low (71% at an Intralipid concentration of 354 mg/dl). This contrasts with the nearly complete recovery of Intralipid at lower Intralipid doses (95% at an Intralipid concentration of 133 mg/dl). This, of course, would not influence the VLDL$_1$-apoB production rate calculations, as Intralipid particles do not contain apoB.

A further issue to consider is whether, after lipolysis by LPL, Intralipid “remnant” particles may have appeared in the VLDL$_1$ fraction, thereby increasing the measured VLDL$_1$-TG concentration and the apparent VLDL$_1$-TG production rate. However, we do not believe that this would have had a substantial effect on calculated VLDL$_1$-TG production rates, for a number of reasons. First, evidence from the literature suggests that for large TG-rich particles, particularly chylomicron-like particles, lipolysis and particle removal from the plasma are likely to occur simultaneously, rather than by sequential mechanisms (24, 25), with the majority of particles removed from the plasma before conversion to smaller VLDL-sized remnant particles (26). Second, as Intralipid contains TG but not apoB, the appearance of Intralipid remnants in the VLDL$_1$ fraction would lead to a disproportionate increase in VLDL$_1$-TG compared with VLDL$_1$-apoB, leading to an increase in the VLDL$_1$-TG/ apoB ratio. We did not observe a significant increase in this ratio during the infusion ($P = 0.21$). Third, if the increase in VLDL$_1$-TG was influenced by the appearance of Intralipid remnant particles, then a positive correlation between Intralipid clearance and VLDL$_1$-TG production would be evident (i.e., increased Intralipid clearance would lead to increased VLDL$_1$-TG production). Instead, a negative relationship between Intralipid clearance and VLDL$_1$-TG production (expressed in mg/h) was observed (i.e., subjects with slow Intralipid clearance also had high VLDL$_1$-TG production) ($r = 0.67, P = 0.04$). Furthermore, the relationship between VLDL$_1$-apoB production, which would be unaffected by the presence of Intralipid remnant particles, and VLDL$_1$-TG production was very strong, with 71% of the variance in the VLDL$_1$-TG production rate explained by the VLDL$_1$-apoB production rate ($r = 0.85, P = 0.002$).

Furthermore, it is important to ascertain whether the results obtained are comparable with data obtained using the gold standard stable isotope tracer method. An internal validation of this method was previously undertaken by Björkegren and colleagues (12) in three subjects. They reported VLDL$_1$-apoB production rates of 20.0, 25.6, and 7.2 mg/h calculated from the Intralipid infusion method, with corresponding rates calculated from a stable isotope method of 23.8, 21.6, and 8.0 mg/h, respectively, indicating that data obtained from the two methods were comparable. In addition, from Table 4, it is clear that the values for VLDL$_1$-apoB production in the present study are of the same order as those obtained from a number of studies that determined VLDL$_1$-apoB production using staple isotope techniques. Determination of VLDL$_1$-TG production rates using stable isotope tracer methods is technically more difficult than determination of VLDL$_1$-apoB production, and the authors are only aware of one group of workers who have evaluated this (7, 27). The values obtained for VLDL$_1$-TG production in our study are of the same order as those published by Adiels et al. (7, 27).

This Intralipid method enabled the Intralipid-TG clearance rate to be calculated in two different ways: from the steady state concentration of Intralipid-TG during the infusion, which we defined as the mean of the final three

Table 3. Correlations between VLDL$_1$-apoB and VLDL$_1$-TG production rates (ranges) calculated in this study (Intralipid method) and in previously published studies using the stable isotope method

| Study                  | Subjects | BMI (kg/m$^2$) | TG (mg/dl) | VLDL$_1$-apoB Production Rates | VLDL$_1$-TG Production Rates |
|------------------------|----------|----------------|-------------|--------------------------------|------------------------------|
|                        | n (Male/Female) |               |             | mg/h                          | mg/h                         |
|                        |           |               |             | mg/kg/day                      | mg/kg/day                    |
| Björkegren et al. (12) | 16 (male) | 20.0–25.8      | 49.6–163.9  | 8.0–23.8                      | 22.9–50.7                    |
| Demant et al. (28)     | 6 (male)  | 20.5–25.0      | 82.0–229.5  | 21.2–51.8                      | 22.9–50.7                    |
| Pietzsch et al. (29)   | 6 (3/3)   | 20.5–25.0      | 62.0–129.4  | 8.5–67.8                       | 22.9–50.7                    |
| Gill et al. (8)        | 16 (8/8)  | 19.6–32.9      | 88.6–279.1  | 8.5–67.8                       | 22.9–50.7                    |
| Adiels et al. (7)      | 17        | 22.4–30.1      | 87.7–229.5  | 22.9–50.7                      | 22.9–50.7                    |
| Zheng et al. (30)      | 5 (female)| 22–27         | 78.9–140.0  | 8.4 ± 3.6$^a$                   | 22–27                        |
| Adiels et al. (27)     | 18        | 22–30         | 59.4–278.2  | 22–27                         | 22–27                        |
| Intralipid method      | 10 (7/3)  | 20.8–34.7      | 39.9–405.8  | 12.0–50.9                      | 4.4–11.5                     |

$^a$Data are mean ± SD.
values if these differed by <13.8% (i.e., two times the CV for the separation of the Intralipid fraction and measurement of the TG), and from the exponential decrease in Intralipid-TG after infusion (15). In subjects in whom a steady-state Intralipid-TG concentration was achieved, the Intralipid-TG clearance rates calculated from the steadystate concentration and from the postinfusion exponential decrease agreed closely (Table 2). However, not all subjects achieved a steady-state Intralipid-TG concentration in 75 min of infusion, and it is not possible to determine whether a steady state was achieved for a given subject until sample analysis was completed. Therefore, in practice, it may be easier to use the postinfusion values to determine Intralipid-TG clearance rates, as this ensures that the Intralipid infusion can be kept as short as necessary to enable the calculation of VLDL1-TG and VLDL1-apoB production rates.

Finally, we sought to determine whether this Intralipid method revealed the physiologically expected differences in TRL kinetics between subjects with differing physical and metabolic profiles. As expected, there were strong positive correlations between fasting TG concentrations and VLDL1-TG production rates, expressed in either absolute terms or normalized according to body mass, and between fasting TG and VLDL1-apoB production, expressed in mg/h, with a strong negative correlation between fasting TG and the Intralipid-TG clearance rates, indicating that those with high TG exhibited a combination of enhanced VLDL1 production and diminished TG clearance. VLDL1-TG FCR in the fasted state (i.e., with no Intralipid present) was ~45% of the Intralipid-TG clearance rate (50.2 ± 5.7 vs. 66.2 ± 11.7 pools/day; Table 2), and there was a very strong correlation between these two variables (r = 0.90, P < 0.0005), indicating that clearance rates for VLDL1 and chylomicron-like particles within an individual are very tightly linked, consistent with the fact that these particles are cleared by the same pathway. The expected positive correlations between indices of body fatness (waist circumference and BMI) and insulin resistance (HOMAIR) and VLDL1-apoB production rates were also observed, in agreement with findings we reported previously using stable isotope tracer methods (8). We also observed significant negative relationships between Intralipid-TG clearance and HOMAIR and waist circumference. Thus, the Intralipid method appears to be sensitive enough to detect physiologically relevant differences in TRL kinetics between individuals across the normal and moderately hypertriglyceremic range.

In conclusion, we have developed a novel method to determine TRL kinetics. The Intralipid method provides a relatively straightforward and cost-effective way of determining VLDL1-TG and VLDL1-apoB production rates and the clearance rate of chylomicron-like particles that does not require specialized equipment, such as a mass spectrometer. We believe that this method will increase the scope for the study of TRL kinetics, particularly in circumstances in which issues related to funding or equipment availability preclude the use of more traditional isotopic tracer methods.

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