methods

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

Iqbal A. R. Al-Shayji,[*,†] Jason M. R. Gill,[1,†] Josephine Cooney,[*] Samira Siddiqui,[*,§] and Muriel J. Caslake[*]

Department of Vascular Biochemistry,[*] and Institute of Diet, Exercise, and Lifestyle, Institute of Biomedical and Life Sciences,[†] University of Glasgow, Glasgow, United Kingdom; and Renal Unit,[§] Glasgow Royal Infirmary, Glasgow, United Kingdom

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 ($VLDL_1$; S_f = 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/m^2] 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 $VLDL_1$ by lipoprotein lipase. Multiple blood samples were taken during and after infusion for separation of Intralipid, $VLDL_1$, and $VLDL_2$ by ultracentrifugation. $VLDL_1$-apolipoprotein B (apoB) and TG production rates were calculated from their linear increases in the $VLDL_1$ fraction during the infusion. Intralipid-TG clearance rate was determined from its exponential decay after infusion. The production rates of $VLDL_1$-apoB and $VLDL_1$-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.**

Supplementary key words Intralipid • triglyceride • apolipoprotein B • production • clearance

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 overproduction of hepatically derived large VLDL [$VLDL_1$; Svedberg flotation rate (S_f) = 60–400] (5, 6). These studies typically use precursors labeled with stable or radioactive isotope tracers 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 $VLDL_1$ 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 $VLDL_1$ 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, Björkegren et al. (12) demonstrated that infusion of Intralipid prevents >90% of $VLDL_1$ catabolism. Therefore, we hypothesized that it would be possible to calculate the production rates of $VLDL_1$-TG and $VLDL_1$-apolipoprotein B (apoB) from the rate of their accumulation during an

Manuscript received 13 November 2007 and in revised form 4 April 2007 and in re-revised form 1 June 2007.

Published, JLR Papers in Press, June 4, 2007.
DOI 10.1194/jlr.D600044-JLR200

Abbreviations: apoB, apolipoprotein B; BMI, body mass index; FCR, fractional catabolic rate; FSR, fractional synthetic rate; $HOMA_{IR}$, homeostasis model assessment insulin resistance; S_f, Svedberg flotation rate; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

[1] To whom correspondence should be addressed.
e-mail: j.gill@bio.gla.ac.uk

infusion of Intralipid. The former would be a measure of lipid production, and the latter would represent the rate of $VLDL_1$ particle production, as there is one apoB molecule per $VLDL_1$ particle. Furthermore, using this approach, it is possible to calculate the rate of Intralipid-TG clearance (a surrogate measure of chylomicron-TG clearance) from either the steady-state Intralipid-TG concentration during infusion (14) or the exponential decay in Intralipid-TG concentration after infusion (15). Here, we report the development and validation of this "Intralipid method" to determine TRL kinetics.

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.

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

Characteristic	Mean	Range
Age (years)	33.5	(20.0–55.0)
BMI (kg/m)	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.

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 $VLDL_1$-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 $VLDL_1$ 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 $VLDL_1$-TG and $VLDL_1$-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/m^2; 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 $VLDL_1$ by LPL and, therefore, enable determination of $VLDL_1$-TG and $VLDL_1$-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 $VLDL_1$, the 0.2 g/kg/h dose would not result in higher calculated production rates of $VLDL_1$-TG or $VLDL_1$-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 ($S_f > 400$) separation from whole plasma

Two milliliters of plasma were overlaid with 4 ml of 1.006 g/ml density solution in ultraclear centrifuge 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

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_f = 60–400) and $VLDL_2$ (S_f = 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-HA; 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.)]. Fasting insulin was analyzed using commercially available ELISA 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 true 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 $VLDL_1$-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 gradient of the linear 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 × 33.8 × 60) 2,147.4 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:

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

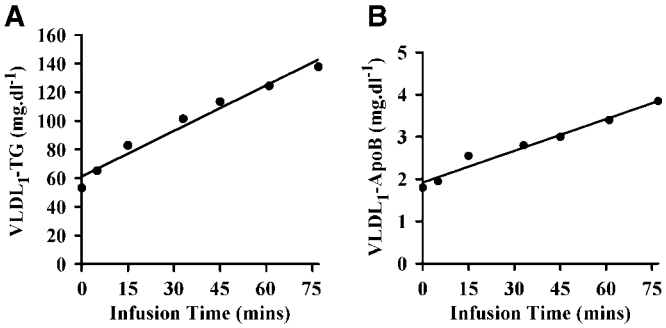

Fig. 1. Changes in $VLDL_1$-triglyceride (TG) (A) and $VLDL_1$-apolipoprotein B (apoB) (B) concentrations (mg/dl) in a female subject (55 years, 84.5 kg) during infusion of 10% Intralipid (0.1 g/kg/h). An initial bolus dose of 20% Intralipid (0.1 g/kg body mass) was given at 0 min. Equations of the line are as follows: for A, y = 1.0589x + 61.191 (R^2 = 0.97); for B, y = 0.0251x + 1.9185 (R^2 = 0.97).

In this method, the FSR of $VLDL_1$-TG and $VLDL_1$-apoB (pools/day) can be calculated from the gradient of the linear increase in their concentrations (mg/dl) over time (min) divided by fasting concentrations (mg/dl) and then multiplied by 60 min and 24 h. However, because the system is in a steady state in fasting conditions, in which the synthesis of $VLDL_1$ equals its clearance, FSR will also be equivalent to the fractional catabolic rate (FCR) in the fasted state (21).

From **Table 2**, subject 3 had $VLDL_1$-TG and $VLDL_1$-apoB fasting concentrations of 49.4 and 1.8 mg/dl, respectively. According to the equation of the line in Fig. 1A, the subject's $VLDL_1$-TG FSR and FCR were (1.0589 ÷ 49.4 × 60 × 24) 30.9 pools/day. Similarly, from Fig. 1B, the $VLDL_1$-apoB FSR and FCR were 19.8 pools/day.

Calculating Intralipid-TG clearance rate. Assuming that all TG clearance is Intralipid-TG clearance, it is possible to determine Intralipid-TG clearance rate 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):

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

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:

$$y = ke^{-bt} \qquad (Eq.\ 3)$$

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

	Fasting Concentrations			Production Rates				Intralipid-TG Clearance Rate		FSR[b]	
Subject[a]	$VLDL_1$-TG	$VLDL_1$-ApoB	$VLDL_2$-ApoB	$VLDL_1$-TG		$VLDL_1$-ApoB		Steady State	Exponential Decay	$VLDL_1$-TG	$VLDL_1$-ApoB
	mg/dl			*mg/h*	*mg/kg/day*	*mg/h*	*mg/kg/day*		*pools/day*		
1	19.9	0.8	1.6	625.0	241.1	17.1	6.6	—[c]	79.8	30.2	22.0
2	20.8	1.1	2.9	719.9	214.6	14.8	4.4	82.6	66.1	25.8	10.3
3	49.4	1.8	6.8	2,147.4	609.9	50.9	14.5	56.9	56.4	30.9	19.8
4	10.2	0.8	0.9	642.3	235.3	12.0	4.4	72.8	96.5	57.8	13.7
5	13.7	1.1	2.2	711.2	309.2	20.7	9.0	—[c]	146.9	56.3	20.0
6	226.8	10.6	7.7	2,563.2	593.2	40.2	9.3	17.3	18.1	6.5	2.2
7	109.4	3.8	3.7	1,228.1	406.5	24.9	8.2	39.8	40.5	9.3	5.4
8	11.5	0.3	3.0	446.0	205.8	16.1	7.4	78.7	80.6	44.7	56.2
9	35.7	1.0	3.0	885.5	322.0	24.1	8.8	43.4	54.3	22.6	21.9
10	28.6	0.5	2.9	798.2	198.5	33.0	8.2	27.2	29.5	17.4	39.1

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 $VLDL_1$-TG and $VLDL_1$-apoB FSR values are equal to the FCR values in the fasted state.
[c] Subject did not reach a steady state.

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

$$b = \frac{\text{clearance rate (ml/min)}}{\text{plasma distribution volume (ml)}} \qquad (Eq.\ 4)$$

Hence,

$$\text{clearance rate (ml/min)} = b \times \text{plasma volume (ml)}$$
$$\text{(pools/day)} = b \times 60 \text{ min} \times 24 \text{ h} \qquad (Eq.\ 5)$$

Intralipid recovery

To assess the recovery of plasma-Intralipid in the Intralipid fraction ($S_f > 400$), 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:

$$\%\ \text{recovery} = \frac{\text{separated Intralipid-TG}}{\text{actual Intralipid-TG}} \times 100 \qquad (Eq.\ 6)$$

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 ($HOMA_{IR}$), production rates of $VLDL_1$-TG (expressed in both mg/h and mg/kg/day) and $VLDL_1$-apoB (expressed in mg/h), Intralipid-TG clearance rate, and $VLDL_1$-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 $HOMA_{IR}$, 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-, $VLDL_1$-, and $VLDL_2$-TG concentrations during and after infusion

Figure 2A shows the mean plasma-, Intralipid-, $VLDL_1$-, and $VLDL_2$-TG responses in 10 subjects during 75 min Intralipid infusion (0.1 g/h/kg body mass). Plasma-TG concentrations were increased to approximately two to three times the fasting value during the infusion. Similarly, mean Intralipid-TG concentrations increased in response to the bolus dose ($P < 0.001$). $VLDL_1$-TG concentrations increased linearly during the infusion ($P < 0.001$), but $VLDL_2$-TG did not change significantly during the course of the infusion ($P = 0.14$).

After stopping the infusion, the plasma- and Intralipid-TG concentrations decreased exponentially (both $P < 0.001$; Fig. 2B). $VLDL_1$-TG continued to increase for ~20 min before plateauing and subsequently decreasing. In subjects in whom the postinfusion period was extended, $VLDL_1$-TG returned to baseline concentrations within 105–135 min (data not shown). The mean $VLDL_2$-TG concentrations remained unchanged for the 75 min postinfusion observation period.

$VLDL_1$-apoB and $VLDL_2$-apoB concentrations during infusion

The mean apoB concentration in the $VLDL_1$ (S_f = 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 $VLDL_2$-apoB concentrations declined significantly ($P < 0.001$)

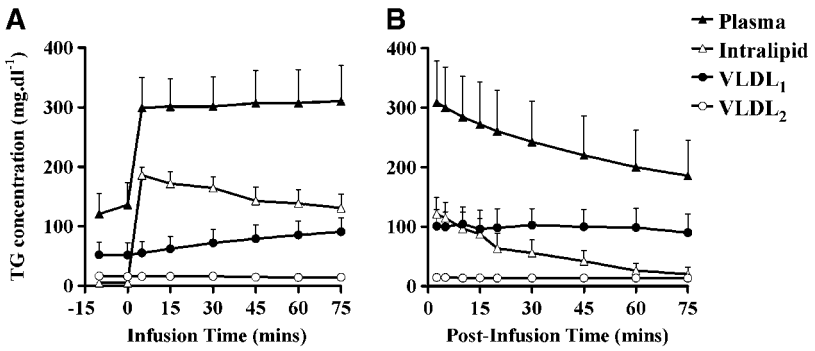

Fig. 2. Plasma-, Intralipid-, $VLDL_1$-, and $VLDL_2$-TG concentrations (mg/dl) during infusion of 0.1 g/kg/h 10% Intralipid (A) and for 75 min after infusion (B). An initial bolus dose of 20% Intralipid (0.1 g/kg body mass) was given at 0 min. Values are means ± SEM; n = 10.

during 75 min of infusion. Total ($VLDL_1$ + $VLDL_2$) VLDL-apoB concentrations increased slightly but significantly ($P < 0.05$) during infusion (**Fig. 3**). There was no significant change in the $VLDL_1$-TG/apoB or $VLDL_2$-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 $VLDL_1$-TG and $VLDL_1$-apoB as well as the clearance rates of Intralipid-TG calculated for each subject (n = 10) as described previously. Fasting $VLDL_1$-TG and $VLDL_1$-apoB and $VLDL_2$-apoB concentrations are also presented.

$VLDL_1$-TG and $VLDL_1$-apoB production rates. The mean ± SEM (range) production rates for $VLDL_1$-TG and $VLDL_1$-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.

$VLDL_1$-TG and $VLDL_1$-apoB FSRs and FCRs. The mean ± SEM (range) $VLDL_1$-TG and $VLDL_1$-apoB FSRs, which are equal to the $VLDL_1$-TG and $VLDL_1$-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.

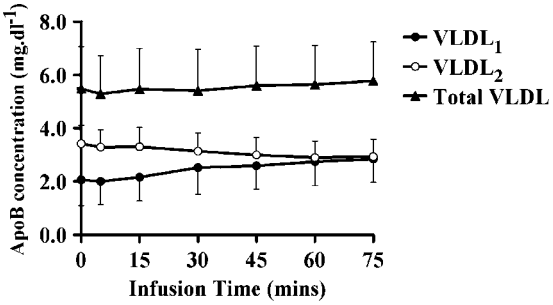

Fig. 3. $VLDL_1$-apoB, $VLDL_2$-apoB, and total VLDL-apoB concentrations (mg/dl) during infusion of 10% Intralipid (0.1 g/kg/h). An initial bolus dose of 20% Intralipid (0.1 g/kg body mass) was given at 0 min. Values are means ± SEM; n = 10.

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 $VLDL_1$-apoB production rates (low dose, 23.8 ± 2.8 vs. high dose, 22.0 ± 1.9 mg/h; $P = 0.21$) or $VLDL_1$-TG production rates (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 $VLDL_1$-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 $VLDL_1$ 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 high Intralipid concentrations (see below). Calculated FSRs for $VLDL_1$-TG (low dose, 30.1 ± 8.8 vs. high dose, 30.4 ± 8.1 pools/day; $P = 0.94$) and $VLDL_1$-apoB (low dose, 28.5 ± 8.7 vs. high dose, 33.4 ± 14.6 pools/day; $P = 0.64$) did not differ between the two doses, and FSRs for $VLDL_1$-TG ($r = 0.88$, $P = 0.05$) and $VLDL_1$-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 ($S_f > 400$) fraction was 95 ± 7% (mean ± SD) for the ~133 mg/dl

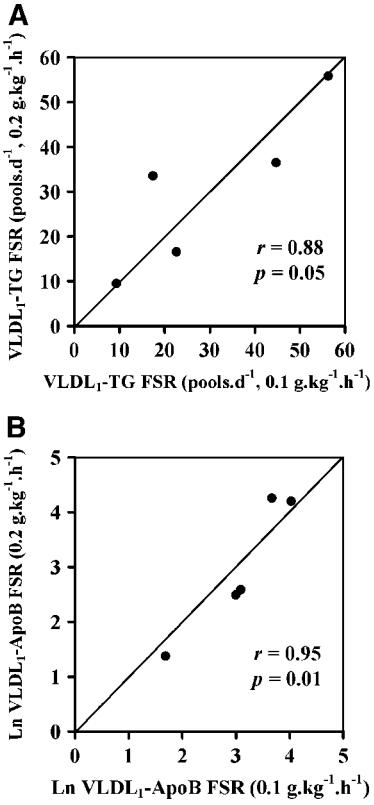

Fig. 4. Scatterplots (with the line of equality) illustrating the agreement between $VLDL_1$-TG fractional synthetic rate (FSR; pools/day) (A) and natural logarithm (Ln) $VLDL_1$-apoB FSR between the low (0.1 g/kg/h) and high (0.2 g/kg/h) doses (B). FSR is equivalent to fractional catabolic rate in the fasted state. n = 5; *r* and *P* values are for Pearson product-moment correlations between variables. $VLDL_1$-apoB FSR is expressed in pools/day before log transformation.

Intralipid-TG concentration and 71 ± 4% for the ~354 mg/dl Intralipid-TG concentration.

Interrelationships between $VLDL_1$-TG and $VLDL_1$-apoB production and TG clearance

Intralipid-TG clearance rate and $VLDL_1$-TG and $VLDL_1$-apoB production rates (expressed in mg/h) were significantly interrelated, with the expected negative correlation between Intralipid-TG clearance and $VLDL_1$-TG ($r = -0.67$, $P = 0.04$) and $VLDL_1$-apoB ($r = -0.69$, $P = 0.03$) production rates and a positive correlation between $VLDL_1$-TG and $VLDL_1$-apoB production rates ($r = 0.85$, $P = 0.002$). There was also a very strong relationship between $VLDL_1$-TG FSR (which equals the $VLDL_1$-TG FCR in the fasted state) and Intralipid-TG clearance rate ($r = 0.90$, $P < 0.005$). The positive correlation between $VLDL_1$-TG and $VLDL_1$-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 $VLDL_1$-TG production rate expressed in mg/kg/day and Intralipid-TG clearance ($r = -0.46$, $P = 0.18$) and between $VLDL_1$-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 $VLDL_1$-TG and $VLDL_1$-apoB production rates expressed in mg/h. $VLDL_1$-TG and $VLDL_1$-apoB production rates correlated strongly and significantly with waist circumference and fasting TG concentration. $VLDL_1$-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 $VLDL_1$-TG ($r = 0.83$, $P = 0.003$) and $VLDL_1$-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 $VLDL_1$-TG and $VLDL_1$-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 $VLDL_1$-TG production and all of these variables remained strong and statistically significant; however, the correlations between $VLDL_1$-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 $VLDL_1$ 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 $VLDL_1$ by LPL (12); therefore, the rates of $VLDL_1$-TG and $VLDL_1$-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 $VLDL_1$ (S_f = 60–400) and $VLDL_2$ (S_f = 20–60) kinetics, found that individual rates of $VLDL_1$-apoB production calculated from the rate of increase of $VLDL_1$-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 $VLDL_1$-TG (i.e., $VLDL_1$ lipid) and $VLDL_1$-apoB (i.e., $VLDL_1$ particle) production as well as the clearance rates of chylomicron-like particles.

The Intralipid method specifically measures the production rate of large $VLDL_1$ rather than total VLDL (i.e., S_f = 20–400). VLDL is a metabolically heterogeneous class of lipoproteins, and it is the larger $VLDL_1$ subclass that competes with chylomicrons/chylomicron-like particles

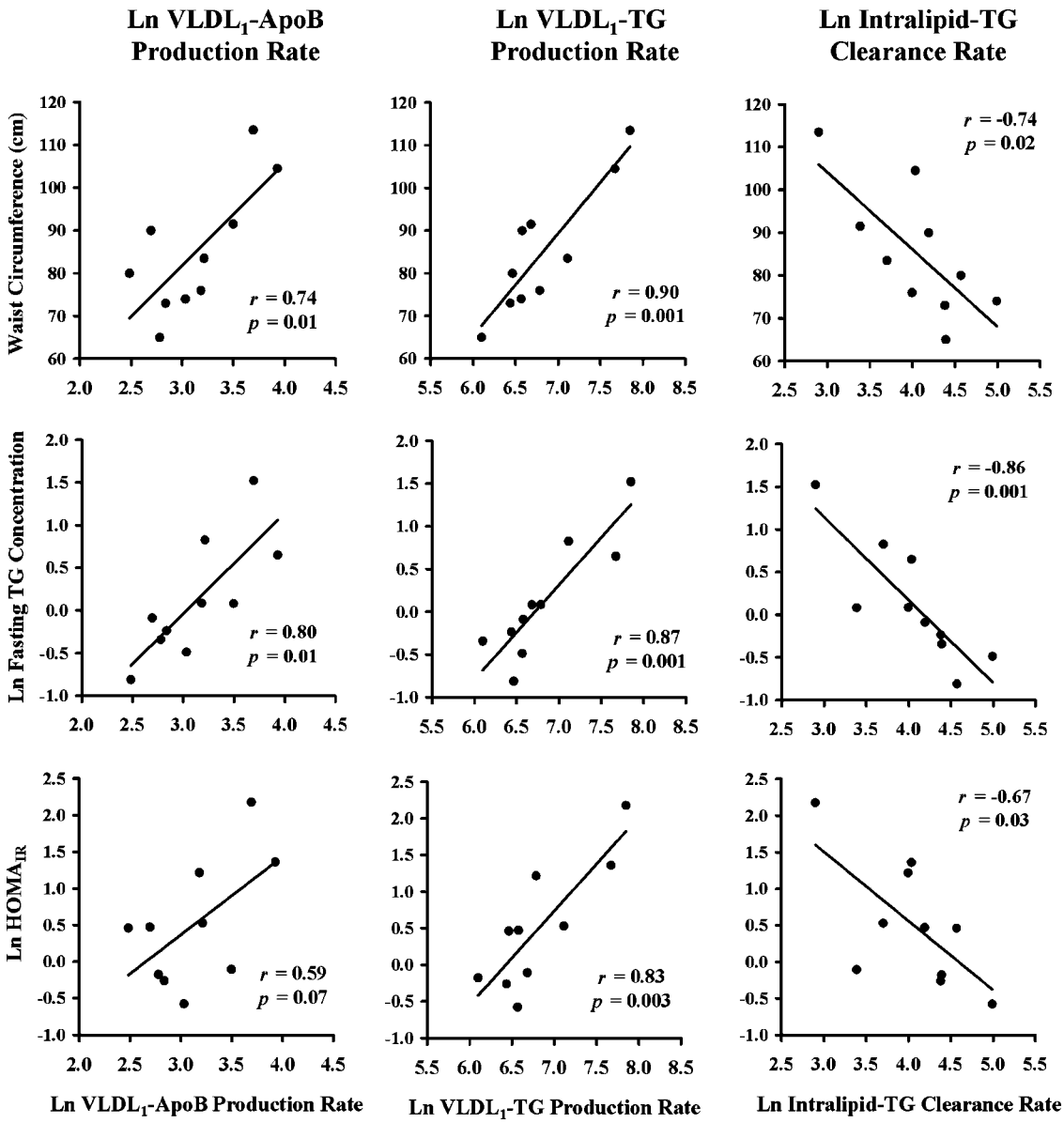

Fig. 5. Scatterplots (with linear regression lines of best fit) illustrating the relationships between the kinetic variables [$VLDL_1$-TG production rate (left), $VLDL_1$-apoB production rate (middle), and Intralipid-TG clearance rate (right)] and subject characteristics: waist circumference (top), fasting TG concentrations (middle), and homeostasis model assessment insulin resistance ($HOMA_{IR}$) (bottom). n = 10; *r* and *P* values are for Pearson product-moment correlations between variables. $VLDL_1$-apoB and $VLDL_1$-TG production rates are expressed in mg/h, and Intralipid clearance rate is expressed in pools/day before log transformation.

for LPL-mediated clearance and would have its clearance blocked by the presence of Intralipid (12). In contrast, catabolism of the smaller $VLDL_2$ 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 $VLDL_2$ is from the catabolism of $VLDL_1$ (the other being direct hepatic production) and this process was blocked by Intralipid infusion, mean $VLDL_2$-apoB concentrations decreased slightly during the infusion, although individual responses were more heterogeneous than those observed with $VLDL_1$-apoB, a finding also reported by Björkegren et al. (12). This heterogeneity in individual $VLDL_2$-apoB responses meant that it was not possible to perform any kinetic analyses using the $VLDL_2$ data.

To validate the calculation of $VLDL_1$-apoB and $VLDL_1$-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 $VLDL_1$-apoB and $VLDL_1$-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 $VLDL_1$: 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 $VLDL_1$-apoB production rate was not changed when a higher (0.2 g/kg/h) Intralipid infusion dose was used. Similarly, FSRs, which

TABLE 3. Correlations between $VLDL_1$-apoB and $VLDL_1$-TG production rates (expressed in mg/kg/day) and subject characteristics

Characteristic	$VLDL_1$-ApoB Production Rate	Ln $VLDL_1$-TG Production Rate
BMI	$r = 0.63$, $P = 0.05$	$r = 0.65$, $P = 0.04$
Waist circumference	$r = 0.45$, $P = 0.19$	$r = 0.70$, $P = 0.02$
Ln fasting TG	$r = 0.54$, $P = 0.11$	$r = 0.79$, $P = 0.01$
Ln $HOMA_{IR}$	$r = 0.40$, $P = 0.25$	$r = 0.79$, $P = 0.01$

Ln, natural logarithm. n = 10; r and P values are for Pearson product-moment correlations between values.

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 4. Comparison of values for $VLDL_1$-TG and $VLDL_1$-apoB production rates (ranges) calculated in this study (Intralipid method) and in previously published studies using the stable isotope method

Study	Subjects			Production Rates			
	n (Male/Female)	BMI	TG	$VLDL_1$-ApoB		$VLDL_1$-TG	
		kg/m^2	*mg/dl*	*mg/h*	*mg/kg/day*	*mg/h*	*mg/kg/day*
Björkegren et al. (12)	16 (male)	20.0–25.8	49.6–163.9	8.0–23.8	—	—	—
Demant et al. (28)	6 (male)	—	88.6–212.6	21.2–51.8	—	—	—
Pietzsch et al. (29)	6 (3/3)	20.5–25.0	62.0–129.4	—	22.9–50.7	—	—
Gill et al. (8)	16 (8/8)	19.6–32.9	88.6–279.1	8.5–67.8	—	—	—
Adiels et al. (7)	17	22.4–30.1	87.7–229.5	—	2.88–12.5	—	107–347
Zheng et al. (30)	5 (female)	22–27	78.9–140.0	—	8.4 ± 5.6[a]	—	—
Adiels et al. (27)	18	22–30	59.4–278.2	—	2.9–12.5	—	107–352
Intralipid method	10 (7/3)	20.8–34.7	39.9–405.8	12.0–50.9	4.4–14.5	446.0–2563.2	199–610

[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 steady-state 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 $VLDL_1$-TG and $VLDL_1$-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 $VLDL_1$-TG production rates, expressed in either absolute terms or normalized according to body mass, and between fasting TG and $VLDL_1$-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 $VLDL_1$ production and diminished TG clearance. $VLDL_1$-TG FCR in the fasted state (i.e., with no Intralipid present) was ~45% of the Intralipid-TG clearance rate (30.2 ± 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 $VLDL_1$ 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 ($HOMA_{IR}$) and $VLDL_1$-TG and $VLDL_1$-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 $HOMA_{IR}$ 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 hypertriglyceridemic 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 $VLDL_1$-TG and $VLDL_1$-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.

I.A.R.A. was supported by the Public Authority of Applied Education and Training from the government of Kuwait. The authors thank Mrs. Dorothy Bedford for technical assistance. The authors also thank Prof. Chris Packard for his valuable advice in the design of the study and the interpretation of the data. Finally, the authors thank Dr. Chris Deighan for medical assistance.

REFERENCES

1. Malloy, M. J., and J. P. Kane. 2001. A risk factor for atherosclerosis: triglyceride-rich lipoproteins. *Adv. Intern. Med.* **47:** 111–136.
2. Tanaka, A., M. Ai, Y. Kobayashi, M. Tamura, K. Shimokado, and F. Numano. 2001. Metabolism of triglyceride-rich lipoproteins and their role in atherosclerosis. *Ann. N. Y. Acad. Sci.* **947:** 207–212.
3. Karpe, F. 1999. Postprandial lipoprotein metabolism and atherosclerosis. *J. Intern. Med.* **246:** 341–355.
4. Schneeman, B. O., L. Kotite, K. M. Todd, and R. J. Havel. 1993. Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. *Proc. Natl. Acad. Sci. USA.* **90:** 2069–2073.
5. Watson, K. E., B. N. Horowitz, and G. Matson. 2003. Lipid abnormalities in insulin resistant states. *Rev. Cardiovasc. Med.* **4:** 228–236.
6. Adiels, M., J. Boren, M. J. Caslake, P. Stewart, A. Soro, J. Westerbacka, B. Wennberg, S. O. Olofsson, C. Packard, and M. R. Taskinen. 2005. Overproduction of VLDL1 driven by hyperglycemia is a dominant feature of diabetic dyslipidemia. *Arterioscler. Thromb. Vasc. Biol.* **25:** 1697–1703.
7. Adiels, M., C. Packard, M. J. Caslake, P. Stewart, A. Soro, J. Westerbacka, B. Wennberg, S. O. Olofsson, M. R. Taskinen, and J. Boren. 2005. A new combined multicompartmental model for apolipoprotein B-100 and triglyceride metabolism in VLDL subfractions. *J. Lipid Res.* **46:** 58–67.
8. Gill, J. M., J. C. Brown, D. Bedford, D. M. Wright, J. Cooney, D. A. Hughes, C. J. Packard, and M. J. Caslake. 2004. Hepatic production of VLDL1 but not VLDL2 is related to insulin resistance in normoglycaemic middle-aged subjects. *Atherosclerosis.* **176:** 49–56.
9. Packard, C. J., T. Demant, J. P. Stewart, D. Bedford, M. J. Caslake, G. Schwertfeger, A. Bedynek, J. Shepherd, and D. Seidel. 2000. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *J. Lipid Res.* **41:** 305–318.
10. Packard, C. J. 1995. The role of stable isotopes in the investigation of plasma lipoprotein metabolism. *Baillieres Clin. Endocrinol. Metab.* **9:** 755–772.
11. Karpe, F., and M. Hultin. 1995. Endogenous triglyceride-rich lipoproteins accumulate in rat plasma when competing with a chylomicron-like triglyceride emulsion for a common lipolytic pathway. *J. Lipid Res.* **36:** 1557–1566.
12. Björkegren, J., C. J. Packard, A. Hamsten, D. Bedford, M. Caslake, L. Foster, J. Shepherd, P. Stewart, and F. Karpe. 1996. Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *J. Lipid Res.* **37:** 76–86.
13. Fisher, R. M., S. W. Coppack, S. M. Humphreys, G. F. Gibbons, and K. N. Frayn. 1995. Human triacylglycerol-rich lipoprotein subfractions as substrates for lipoprotein lipase. *Clin. Chim. Acta.* **236:** 7–17.
14. Rang, H. P., M. M. Dale, J. M. Ritter, and P. K. Moore, editors. 2003. Pharmacology. Churchill Livingstone, London.
15. Rössner, S. 1974. Studies on an intravenous fat tolerance test. Methodological, experimental and clinical experiences with Intralipid. *Acta Med. Scand. Suppl.* **564:** 1–24.
16. Gill, J. M., and A. E. Hardman. 2003. Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets (Review). *J. Nutr. Biochem.* **14:** 122–132.
17. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
18. Lindgren, F. T., L. C. Jensen, and F. T. Hatch. 1972. The isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism. G. J. Nelson, editor. John Wiley and Sons, New York. 181–274.

19. Howdieshell, T. R., N. Bhalla, J. T. DiPiro, T. Kuske, and R. Baisden. 1995. Effects of free glycerol contained in intravenous fat emulsion on plasma triglyceride determination. *JPEN J. Parenter. Enteral Nutr.* **19:** 125–126.
20. Packard, C. J., A. Munro, A. R. Lorimer, A. M. Gotto, and J. Shepherd. 1984. Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *J. Clin. Invest.* **74:** 2178–2192.
21. Foster, D. M., P. H. Barrett, G. Toffolo, W. F. Beltz, and C. Cobelli. 1993. Estimating the fractional synthetic rate of plasma apolipoproteins and lipids from stable isotope data. *J. Lipid Res.* **34:** 2193–2205.
22. Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* **28:** 412–419.
23. Packard, C., and J. Shepherd. 1997. Lipoprotein heterogeneity and apolipoprotein B metabolism. *Arterioscler. Thromb. Vasc. Biol.* **17:** 3542–3556.
24. Olivecrona, G., and T. Olivecrona. 1998. Clearance of artificial triacylglycerol particles. *Curr. Opin. Clin. Nutr. Metab. Care.* **1:** 143–151.
25. Hultin, M., R. Savonen, and T. Olivecrona. 1996. Chylomicron metabolism in rats: lipolysis, recirculation of triglyceride-derived fatty acids in plasma FFA, and fate of core lipids as analyzed by compartmental modelling. *J. Lipid Res.* **37:** 1022–1036.
26. Karpe, F., T. Olivecrona, A. Hamsten, and M. Hultin. 1997. Chylomicron/chylomicron remnant turnover in humans: evidence for margination of chylomicrons and poor conversion of larger to smaller chylomicron remnants. *J. Lipid Res.* **38:** 949–961.
27. Adiels, M., M. R. Taskinen, C. Packard, M. J. Caslake, A. Soro-Paavonen, J. Westerbacka, S. Vehkavaara, A. Hakkinen, S. O. Olofsson, H. Yki-Jarvinen, et al. 2006. Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia.* **49:** 755–765.
28. Demant, T., C. J. Packard, H. Demmelmair, P. Stewart, A. Bedynek, D. Bedford, D. Seidel, and J. Shepherd. 1996. Sensitive methods to study human apolipoprotein B metabolism using stable isotope-labeled amino acids. *Am. J. Physiol. Endocrinol. Metab.* **270:** E1022–E1036.
29. Pietzsch, J., B. Wiedemann, U. Julius, S. Nitzsche, S. Gehrisch, S. Bergmann, W. Leonhardt, W. Jaross, and M. Hanefeld. 1996. Increased clearance of low density lipoprotein precursors in patients with heterozygous familial defective apolipoprotein B-100: a stable isotope approach. *J. Lipid Res.* **37:** 2074–2087.
30. Zheng, C., K. Ikewaki, B. W. Walsh, and F. M. Sacks. 2006. Metabolism of apoB lipoproteins of intestinal and hepatic origin during constant feeding of small amounts of fat. *J. Lipid Res.* **47:** 1771–1779.