methods

A stable isotope method using a [$^2H_5$]glycerol bolus to measure very low density lipoprotein triglyceride kinetics in humans

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Abstract **We have developed a method using a bolus of [$^2H_5$]glycerol to determine parameters of VLDL-triglyceride (VLDL-TG) turnover and have compared the data to that obtained using simultaneously a bolus of [2-^{3}H]glycerol in six young normolipidemic men. No measurable enrichment was found after 12 h for [$^2H_5$]glycerol; therefore, we could only perform a monoexponential analysis of its data. No differences in fractional catabolic rate (FCR) were seen when comparing the multicompartmental modeling of [2-^{3}H]glycerol data (modeled over 48 h) either to the monoexponential analyses of the [2-^{3}H]glycerol or that of the [$^2H_5$]glycerol data. The two monoexponential approaches were highly correlated ($r = 0.96$ for FCR), however, FCR was 18% higher with the [$^2H_5$]glycerol than with the [2-^{3}H]glycerol data ($P < 0.003$). In all six subjects, a 10-h infusion of [1-^{13}C]acetate was started at the same time as the glycerol boluses were given. In two men we were able reliably to detect VLDL-TG-fatty acid enrichment. The measurement of FCR in these two subjects using the mass isotopomer distribution analysis (MIDA) approach was in good agreement (within 10%) with FCRs determined with the labeled glycerol methods. In conclusion, our results have shown that results obtained with the [$^2H_5$]glycerol bolus were highly correlated with those obtained with the [2-^{3}H]glycerol, but the FCRs were slightly higher with the former. We have also demonstrated that FCRs determined from monoexponential modeling were in good agreement with those determined from the multicompartmental modeling of the TG-glycerol data.**—Lemieux, S., B. W. Patterson, A. Carpentier, G. F. Lewis, and G. Steiner. **A stable isotope method using a [$^2H_5$]glycerol bolus to measure very low density lipoprotein triglyceride kinetics in humans.** *J. Lipid Res.* **1999.** 40: **2111–2117.**

Supplementary key words stable isotopes • radioactive isotopes • glycerol • methods

Hypertriglyceridemia is associated with an increased risk of cardiovascular disease (1). For this reason, the metabolism of plasma triglyceride (TG) has been studied widely in humans. Steady state levels of plasma TG reflect the balance between the secretion and clearance of the TG-carrying lipoproteins in the circulation. In the fasting plasma these are primarily the endogenously produced TG-rich lipoprotein family, the very low density lipoproteins (VLDL). The VLDL-TG turnover provides information on TG metabolism that is not provided by a simple measurement of plasma TG concentration.

The most widely used and documented method to measure VLDL-TG turnover rate relies on the injection of a bolus of a radioactive tracer, generally [2-^{3}H]glycerol. This endogenously labels the VLDL-TG glycerol. Following the decline of VLDL-TG glycerol specific activity (SA) over a period of 24 to 48 h allows VLDL-TG kinetics to be calculated by modeling the data using either a single compartment, monoexponential approach (2–4) or a multicompartmental approach (5–8). Although the amount of radioactivity exposure is quite small and is considered to be safe, the injection of a radioactive substance, particularly if it were to be done on multiple occasions, is considered a disadvantage for this method. The necessity to obtain samples over a 24–48 h period of metabolic control in studies that depend on multicompartmental modeling is also a limitation of this approach.

Stable isotopically labeled tracers can also be used to study the kinetics of lipids and lipoproteins (9–15). VLDL-TG can be labeled in its fatty acid or glycerol moieties by administering the appropriate stable isotopically labeled precursor. The enrichment of the VLDL-TG can

Abbreviations: TG, triglyceride; FCR, fractional catabolic rate; FAME, fatty acid methyl ester; MIDA, mass isotopomer distribution analysis; SA, specific activity; GC–MS, gas chromatography–mass spectrometry; TTR, tracer to tracee ratio; FSR, fractional synthetic rate; ASR, absolute synthetic rate.

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then be determined by gas chromatography–mass spectrometry (GC–MS). [^{13}C]acetate infusions have been used to label the fatty acids in VLDL-TG that are newly made by the liver and from this to determine both total VLDL-TG production and the proportion of this that is attributable to de novo lipogenesis (12, 13, 15). An infusion of labeled glycerol has also been used by Siler et al. (15) to study the kinetics of VLDL-TG. To our knowledge, the use of stable isotopically labeled glycerol given as a bolus has not yet been described for the study of the kinetics of VLDL-TG. The development of such a method could provide a useful tool for those studying VLDL-TG metabolism in humans.

This study was undertaken to compare the method using a bolus injection of [$^{2}H_5$]glycerol to two other established methods that are used to measure VLDL-TG kinetics: *1*) a radioactive tracer ([2-^{3}H]glycerol bolus) and *2*) a constant 10-h infusion of [1-^{13}C]acetate with the mass isotopomer distribution analysis (MIDA).

EXPERIMENTAL DESIGN

Subjects

Six non-obese young men were recruited by advertising in the University of Toronto newspapers. Subjects were healthy and were not taking any medication. Their alcohol consumption was at most moderate (no more than two alcoholic beverages per day). The Toronto Hospital Committee for Research on Human Subjects approved the study and all subjects gave their written informed consent prior to their participation in the study.

Study design

For 2 days prior to the study, the subjects refrained from drinking alcohol and ate prepackaged food that had been prepared by a dietician to conform to an American Heart Association phase 1 weight maintenance diet. After a 12-h fast, they were admitted to the Metabolic Investigation Unit of the Toronto Hospital. A cannula was inserted in each forearm, one for sampling and one for infusion. After taking a baseline blood sample (at approximately 8 am), each subject was simultaneously given a bolus of 200 μCi of [2-^{3}H]glycerol and a bolus of 100 μmol/kg of [1,1,2,3,3-^{2}H]glycerol (98% enriched; Cambridge Isotope Laboratories, Andover, MA). Immediately thereafter a 10-h infusion (Harvard Pump, Harvard Apparatus, Natick, MA) of 2 μmol/kg·min of [1-^{13}C]acetate (99% enriched; Cambridge Isotope Laboratories, Andover, MA) was started. Subjects continued fasting for 10 h during which the [1-^{13}C]acetate was infused. After this 10-h period, until the end of the study, they ate an isocaloric diet that contained only 10% of energy from fat and was given as 6 meals/day. This was done to minimize the formation of chylomicrons from ingested fat.

Blood sampling and ultracentrifugation

Blood was drawn into Vacutainer tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ) containing Na_2EDTA (4 mmol/L) at 0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 12, 16, 20, 24, 32, 40, and 48 hours after the administration of the tracers and placed immediately on ice. The blood was centrifuged within 30 min (1500 rpm, for 20 min at 4°C) to separate the plasma. The plasma was overlaid with a NaCl solution (d 1.006 g/ml) and ultracentrifuged at 4°C for 30 min at 7000 rpm in a 70.1 Ti Rotor (Beckman Instruments, Inc., Palo Alto, CA). After removing the top ($S_f > 400$) layer, the $S_f < 400$ fraction was overlaid with a solution of d 1.006 g/ml and spun at 39,000 rpm in a 70.1 Ti Rotor for 16 h at 4°C. The top layer of this fraction (S_f 20–400, VLDL) was then removed for further analysis.

Lipid assays

Cholesterol and TG were measured enzymatically using commercially available kits (Boehringer Mannheim Biochemicals, Laval, Québec).

Sample preparation for the determination of VLDL-TG enrichment or specific activity

A total lipid extract was obtained from the VLDL fraction using chloroform–methanol 2:1 according to the procedure of Folch et al. (16). A known aliquot of the lipid extract was used to determine [2-^{3}H]glycerol specific activity while another known aliquot of the lipid extract was used to determine TG-glycerol and TG-fatty acid enrichment with GC/MS.

Determination of the VLDL-TG -glycerol specific activity (SA)

The aliquot for specific activity determination was dried under N_2 and redissolved in isopropanol. Zeolite was added to this in order to remove phospholipids. After centrifugation, a known aliquot of the supernatant was put in a counting vial and dried. Aqueous counting scintillant (Amersham, Oakville, Ontario) was then added to the dried extract and samples were counted in a liquid scintillation counter with corrections for counting efficiency being made (Beckman, LS6500, multi-purpose scintillation counter). Another known aliquot of the Zeolite-treated lipid extract was used to measure its TG concentration.

Determination of glycerol and fatty acid enrichments with GC/MS

The lipid components (cholesteryl esters, TG, cholesterol, free diacylglycerol, free fatty acids, and phospholipids) of the VLDL lipid extract were resolved as bands by thin-layer chromatography on 20 × 20 cm silica gel plates (Fisher, #06-600A Gel G TLC plates) using heptane–isopropyl ether–acetic acid 80:20:2 (17). The silica gel containing the TG band was scraped off and chloroform–methanol was added. After centrifugation to spin down the silica gel, a solution of 10% acetyl chloride–methanol was added to the supernatant. The solution was incubated at 70°C for 30 min and then dried. Hexane was added to dissolve fatty acid methyl esters (FAME). Glycerol is not dissolved in hexane and remains bound to the glass vial. A solution of 5% heptafluorobutyric anhydride in ethyl acetate (1:19) was added to derivatize glycerol and the solution was incubated at 70°C for 30 min. After evaporating the solution, ethyl acetate was added before injecting into the GC. Relative concentrations of individual fatty acids in the VLDL fraction were determined by GC. The FAME were analyzed by GC/MS with electron impact ionization. The GC (Hewlett-Packard Model 5890, Palo Alto, CA) was equipped with a 30 m × 0.25 mm, 0.25 m film Omegawax 250 capillary column (Supelco, Bellefonte, PA) interfaced to a model 5971 quadrupole mass selective detector (Hewlett-Packard) (18). For glycerol analyses, negative chemical ionization GC/MS was performed (19) using a 15 m × 0.25 mm, 0.25 m film Rtx-200 capillary column (Restek, Bellefonte, PA) on a model 5988 quadrupole GC/MS (Hewlett-Packard).

We analyzed the methyl ester of palmitate (16:0; *m/z* 270) and its isotopomers m + 1 and m + 2 (*m/z* 271,272). We also determined the isotopomers distributions of stearate (*m/z* 298, 299, 300) and of oleate (*m/z* 296, 297, 298). Glycerol (*m/z* 680) and its isotopomer m+5 (*m/z* 685) were also measured. The glycerol tracer:tracee ratio (TTR) was determined by calibration of measured m+5/m+0 ratios for standards of known isotopic enrichment.

Calculation of VLDL-TG turnover parameters

Methods used to determine parameters of VLDL-TG turnover with *1*) radiolabeled glycerol and *2*) stable isotopically labeled glycerol tracers were very similar. In the first case, the SA of VLDL-TG glycerol was determined whereas in the second case, the ratio of m+5/m+0 (tracer-to-tracee ratio or TTR) of VLDL-TG glycerol was measured. We measured parameters of VLDL-TG turnover from the log linear phase of decline in SA of VLDL-TG versus time, as originally described by Reaven et al. (20). Points chosen were from the peak of enrichment down to the last points which appeared, by visual inspection, to be on the line. The calculated slope of the linear phase corresponded to the fractional catabolic rate (FCR). The absolute secretion rate of VLDL-TG was obtained by multiplying the FCR by the VLDL-TG pool size (mean VLDL-TG concentration times the assumed plasma volume, i.e., 37 ml/kg body weight). Curves of SA ([2-^{3}H]glycerol) versus time were also modeled using a multi-compartmental model to determine parameters of TG turnover rate according to Zech et al. (5). Briefly, the model includes a plasma glycerol subsystem, which is assumed to be a two compartment system with rate constant for the glycerol system fixed at values as described by Zech et al. (5), based on bolus glycerol kinetic study reported by Malmendier, Delcroix, and Berman (21). A portion of the plasma tracer passes through a delay compartment and emerges as VLDL-TG, which turns over and returns back to plasma. The VLDL FCR is adjusted to provide the best fit of the data and the absolute synthetic rate (ASR) is calculated by multiplying the FCR by the VLDL-TG pool.

We also attempted to model stable isotope TTR values over the 48-h period using the model developed by Zech et al. (5). However, in four out of six subjects, after TTR values were too small to be accurately measured at the later stage of the study to provide adequate modeling. For that reason, VLDL-TG kinetic values derived from multi-compartmental modeling of the [$^2H_5$]glycerol data will not be presented in this report.

Determination of the VLDL-TG turnover rate from the [1-^{13}C] acetate tracer was performed according to the method described by Aarsland, Chinkes, and Wolfe (13). The calculations are based upon the MIDA approach (12). We therefore refer to this method of measuring VLDL-TG kinetics as the MIDA approach. We first calculated the rate at which palmitate synthesized de novo is secreted as VLDL-TG. For that purpose, the enrichment (p) of the intrahepatic precursor pool for fatty acids synthesis (hepatic acetyl-CoA) was determined according to the MIDA method as previously described (22). The fractional synthetic rate (FSR) was expressed as a percentage and represents the fraction of the VLDL-TG-palmitate pool per unit of time that is newly synthesized. It was calculated according to Chinkes et al. (23). To obtain the absolute rate of de novo synthesized fatty acids, the FSR was multiplied by the pool size of VLDL-TG- palmitate. This was estimated by multiplying the mean concentration of VLDL-TG pool measured during the 10-h acetate infusion by the amount of palmitate in the VLDL-TG relative to the total VLDL-TG fatty acids.

In order to obtain the total rate of VLDL-bound palmitate secreted, we calculated the % of VLDL-bound palmitate that is derived from de novo produced palmitate (% de novo VLDL palmitate). This was determined from the extent of dilution, which occurs between the precursor and the product. The formula as described by Aarsland et al. (13) is:

$$\%\ \text{de novo VLDL palmitate} = (\text{plateau enrichment of m + 1 palmitate}/(8 \times p))/(1 - p)^7$$

The total rate of VLDL-bound palmitate secretion was then calculated by dividing the absolute rate at which de novo synthesized palmitate is secreted as VLDL-TG by the % of VLDL-bound palmitate that is derived from de novo produced palmitate.

Attempts were made to measure the secretion rates of stearate, oleate, linoleate, and palmitoleate according to calculations proposed by Aarsland et al. (13). However, for most of our subjects, stearate and oleate enrichment were too low to perform reliable calculations. Therefore the total VLDL-TG secretion was derived from the palmitate secretion rate. To obtain the amount of TG produced, we first divided the secretion of palmitate in mmol by the percentage of palmitate in VLDL-TG fatty acids to estimate the total secretion of VLDL-TG fatty acids in mmol. We then divided by 3 to transform the mmol of fatty acids into mmol of TG. The FCR of the VLDL-TG was obtained by dividing the rate of secretion of VLDL-TG by its pool size.

Statistical analyses

Comparisons of TG turnover parameters obtained using the different methods were performed using analysis of variance (ANOVA) for repeated measures followed by paired *t*-test to determine specifically the significance of the difference between two groups. Spearman correlation coefficients were computed to determine the correlation between each analytical approach and regression equations were determined. All statistical analyses were performed using the SAS software.

RESULTS

The mean age of the six men participating in this study was 23.0 ± 2.2 years (mean ± SD). The mean of their body mass index was 22.4 ± 1.6 kg/m^2 and they had normal plasma lipids (plasma TG: 1.14 ± 0.59 mm, plasma cholesterol: 3.94 ± 0.81 mm, VLDL-TG: 0.72 ± 0.45 mm). Their VLDL-TG levels were stable during the study. The coefficient of variation of VLDL-TG levels was calculated for each subject, in the fasting state (0–10 h) and throughout the duration of the study (0–48 h). Plasma VLDL-TG concentrations varied by 35 ± 15% when subjects were in the fasting state and by 31 ± 8% over the entire 48-h study period. Therefore, the ingestion of the low fat diet at selected time-points during the turnover study was not associated with a greater variation in TG levels than that observed in the fasting state.

Figure 1 shows the SA for the radioactively labeled VLDL-TG glycerol, and the TTR for the stable isotopically labeled VLDL-TG glycerol, versus time in each of the subjects studied. For the latter, only those samples with reliably detectable enrichment are plotted. In general, this was only up to 12 to 20 h after the [$^2H_5$]glycerol bolus injection. The mean of the peak absolute value for SA was 768.8 ± 304.6 dpm/mg of TG and 0.82 ± 0.29% for TTR. We calculated the efficiency of labeling for both [2-^{3}H]- and [$^2H_5$]glycerol. The mean efficiency of labeling for [2-^{3}H]glycerol was $1.59 \times 10^{-5} \pm 0.63 \times 10^{-5}$ and corresponded to the peak SA value (converted in dpm/mg of TG glycerol) divided by the amount of radioactivity given in the bolus (200 μCi ^{3}H or 4.4×10^8 dpm ^{3}H). For [$^2H_5$]glycerol, the efficiency of labeling was $1.29 \times 10^{-5} \pm 0.53 \times 10^{-5}$ and was obtained by dividing the peak TTR value divided by the amount of [$^2H_5$]glycerol given in the bolus. To enable visual comparison between the two ap-

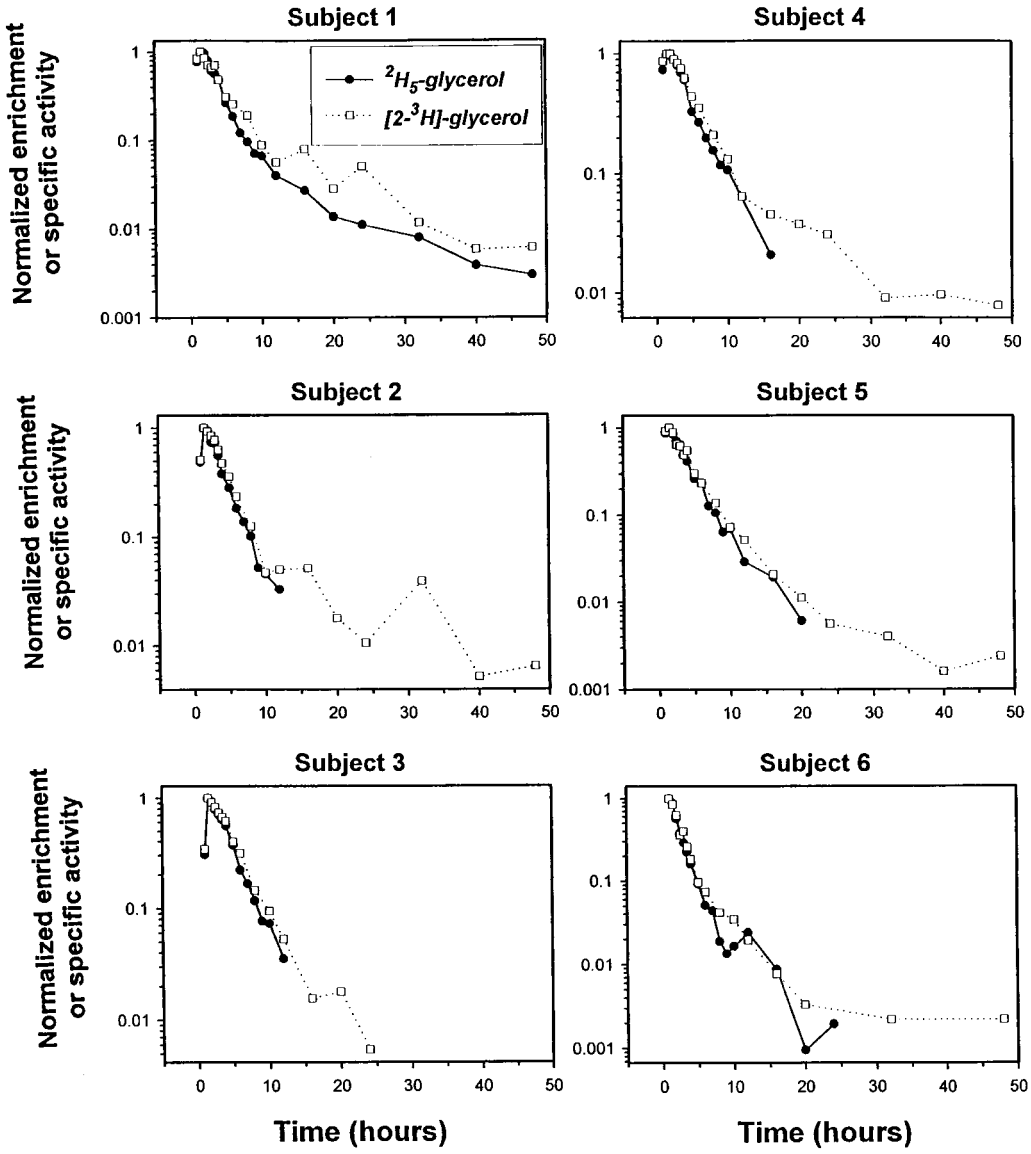

Fig. 1. The time course of changes in the enrichment or specific activity (SA) of VLDL-TG glycerol after a simultaneous bolus intravenous injection of [$^2H_5$]glycerol and [2-^{3}H]glycerol, respectively. Data are presented for each person studied and are given as a proportion of the maximum enrichment or specific activity.

proaches, SA and TTR values were normalized and expressed as a percentage of the peak value, plotted on a log scale. Figure 1 shows the rate of decline of the SA and the TTR to be very similar. In addition, the times at which the highest values for the SA or TTR were seen in the VLDL-TG were identical for each study participant.

The first three columns of **Table 1** show individual and mean data of FCR as determined by: *1*) the multicompartmental modeling of VLDL-TG [2-^{3}H]glycerol SA values; *2*) the monoexponential decrease of VLDL-TG [2-^{3}H]glycerol SA values; *3*) the monoexponential decrease of VLDL-TG glycerol TTR ([$^2H_5$]glycerol). The analysis of variance for repeated measurements revealed a significant effect of the approach used ($P = 0.04$). By paired analysis, we found that the monoexponential analysis of the [$^2H_5$]glycerol data gave a higher FCR value ($P = 0.003$) than did monoexponential analysis of [2-^{3}H]glycerol data. The difference between these two methods was 18.7 ± 8.9%. However, FCR derived from the monoexponential analysis of the [$^2H_5$]glycerol was closely correlated to FCR obtained from the monoexponential analysis of [2-^{3}H]glycerol data ($r = 0.96$). No significant differences were found between either monoexponential approach and the multicompartmental approach (a difference of 1.8 ± 11.5%). In addition, FCRs derived from the monoexponential analysis of [2-^{3}H]glycerol were also well correlated to FCRs derived from the multi-compartmental modeling of VLDL-TG [2-^{3}H]glycerol ($r = 0.85$). Two participants (1 and 4) had sufficient palmitate enrichment to permit analysis of their data by the MIDA approach. As indicated in the Experimental Design section, we derived parameters of VLDL-TG kinetics exclusively from data obtained on palmitate secretion as the enrichment of other fatty acids were too low. The fourth column of Table 1

TABLE 1. Fractional catabolic rate (FCR) calculated by the four approaches described

	Radioactive Isotope [2-^{3}H]glycerol		Stable Isotope	
			[$^2H_5$]glycerol	[1-^{13}C]acetate
Subject	Multicompartmental	Monoexponential	Monoexponential	MIDA
		FCR (pools/h)		
1	0.270	0.278	0.378	0.248
2	0.391	0.326	0.359	nd
3	0.329	0.291	0.335	nd
4	0.276	0.262	0.301	0.284
5	0.276	0.290	0.339	nd
6	0.424	0.484	0.559	nd
Mean ± SD	0.328 ±0.067	0.322 ±0.082	0.378 ±0.092[a]	

[a] Significantly higher than the value obtained with [2-^{3}H]glycerol monoexponential, $P = 0.003$.

shows their FCR obtained with MIDA. Both were similar (+2.8% in one case and −8.1% in the other) to the rates obtained with labeled glycerol.

DISCUSSION

Historically, the most commonly used method to assess TG turnover in humans has been to inject radioactively labeled glycerol ([2-^{3}H]glycerol) as an intravenous bolus, followed by frequent sampling over a period of time long enough to follow the monoexponential decay of VLDL-TG glycerol SA or to perform the multi-compartmental modeling of the changes in SA. However, even if this method is the most commonly used, it has its limitations. For example, the multi-compartmental model suggested initially by Zech et al. (5) assumes the plasma glycerol to be a two-compartment system with rate constants fixed at values based on bolus glycerol kinetics. Hence, the kinetics of plasma glycerol are assumed to be the same for each subject. However, this need not be the case. Ideally, plasma [2-^{3}H]glycerol should be measured for each subject in order to derive individualized rate constant values for the plasma glycerol subsystem.

Recently, there has been a growing interest in using stable, rather than radioactive, isotopically labeled tracers (9–15). Aarsland and colleagues (13) have proposed that a 10-h infusion of [1-^{13}C]acetate coupled with measurement of VLDL fatty acid enrichment by GC-MS may be used to determine VLDL-TG secretion rate. To date, no study has simultaneously compared parameters of VLDL-TG kinetics derived from data obtained after a bolus of [2-^{3}H]glycerol to those obtained by methods using stable isotopically labeled tracers. In this study we have measured parameters of VLDL-TG turnover using three tracers simultaneously: a bolus of [2-^{3}H]glycerol, a bolus of [$^2H_5$]glycerol, and an infusion of [1-^{13}C]acetate. The [$^2H_5$]glycerol approach produced TG-glycerol enrichment that was reliably measurable for only about 12 h after the bolus injection. During that time, the enrichment of the [$^2H_5$]glycerol-labeled TG declined in a monoexponential manner. Therefore we compared the kinetics, obtained with the monoexponential modelling of the [$^2H_5$]glycerol-labeled TG data to the kinetics obtained with the monoexponential modeling of the [2-^{3}H]glycerol-labeled TG data. By paired analysis we found that the FCR obtained by monoexponential analysis of the [$^2H_5$]glycerol data was 18% greater than that obtained by monoexponential analysis of the [2-^{3}H]glycerol-labeled TG data. Among the possible explanations for this difference are isotope effects due to alterations in the tracer resulting from the five deuteria, or loss of the ^{2}H during stages of intermediary metabolism which affects the integrity of the tracer (B. W. Patterson, personal observation). Although this was significant statistically, it is less than the differences that are generally considered to be biologically important when comparing different groups of people or studying the effects of physiologic perturbations in them.

These studies also gave us the opportunity to compare the VLDL-TG kinetics obtained by multicompartmental modeling of the data obtained over 48 h versus monoexponential analysis of the data obtained over 12 h after a [2-^{3}H]glycerol injection. We found that VLDL-TG FCR derived from the monoexponential decrease of the SA curve of VLDL-TG [2-^{3}H]glycerol was well correlated with the FCR obtained from 48-h modeling of VLDL-TG SA ($r = 0.85$). We also observed that there were no differences between the FCRs obtained from monoexponential analysis of the glycerol stable isotope data and those obtained by multicompartmental analysis of the radioactive glycerol data. Grundy et al. (6) reported a weaker correlation ($r = 0.55$) between the monoexponential and the multicompartmental analyses of their data after an injection of [2-^{3}H]glycerol methods in non-obese individuals. However, their subjects included individuals with plasma TG levels as high as 8 mmol/l whereas all our subjects were normotriglyceridemic. Moreover, in a companion paper of theirs (5) the specific FCR information (unfortunately without their fasting plasma TG levels) is given for two of their participants and multicompartmental versus monoexponential analysis gave FCRs within 10% of each other.

Our results also indicated that curves of VLDL-TG glycerol SA and of VLDL-TG glycerol TTR were very similar when they were normalized by setting the highest value at 100%. Within each subject, the maxima for the tracer enrichment of the triglyceride-[$^2H_5$]glycerol and for the SA of the [2-^{3}H]glycerol were observed at the same time and declined with a similar pattern. As we were unable to detect

m+5 enrichment of glycerol reliably beyond 12–20 h after the bolus of [$^2H_5$]glycerol was injected, and as the decline in enrichment over this time was monoexponential, we were only able to use the monoexponential approach to model the kinetics obtained with this stable isotope. It is possible that better enrichments would be obtained in subjects with a slower FCR, such as those who are obese and/or insulin-resistant and/or hypertriglyceridemic (7, 24, 25). The use of GC-IRMS could improve the ability to detect very low enrichment; however, this method requires the use of isotopes labeled with ^{13}C tracer for glycerol. Previous work (5) has suggested that ^{13}C tracer would recycle to a greater extent than the deuterated tracer (hydrogens are lost in intermediary metabolism stages, but C skeletons recycle).

Some may consider the difficulty to detect m+5 enrichment of glycerol at later time points as a limitation to the use of [$^2H_5$]glycerol. However, we have noted, as discussed above, that any differences in the kinetics obtained with the monoexponential analysis of the data using [$^2H_5$]glycerol and the kinetics obtained with either the monoexponential or the multicompartmental analysis of the data obtained when [2-^{3}H]glycerol were small. At present there is no way to know with certainty which of the approaches gives the "truest" FCR of VLDL-TG. However, in view of their close agreement, it is reasonable to suggest that the calculation of VLDL-TG FCR based on the monoexponential decrease of TG-[$^2H_5$]glycerol values may be used reliably to "rank" subjects or to follow intra-individual changes.

In two individuals we were also able to conduct a preliminary comparison of the kinetics obtained from TG-glycerol data to those obtained by the MIDA approach. The latter examines the enrichment of VLDL-TG-fatty acids during and after an infusion of [1-^{13}C]acetate. The MIDA approach is interesting because of its shorter duration (10 h) and also because it provides additional information on TG kinetics, i.e., the proportion of de novo versus pre-formed fatty acids contributing to total VLDL-TG secretion. In our studies we were able to detect enrichment only in these two people and only in their VLDL-TG palmitate. This may reflect the fact that our participants did not have a sufficiently high rate of de novo TG synthesis. Perhaps this was because they were normolipidemic and de novo lipogenesis was not artificially stimulated by feeding high carbohydrate diets or by infusing glucose, as previously done by others (12), during the study. We adopted a dose of labeled acetate which has been commonly used by others (13, 23). However, it would be worthwhile to verify whether an increase in the amount of [1-^{13}C]acetate administered during the infusion could improve the capacity to detect sufficient enrichment.

Using MIDA, we estimated the total VLDL-TG secretion rates from the secretion rate of VLDL-TG-palmitate and a measure of the relative palmitate content of the VLDL-TG. In the two individuals in whom it could be assessed, we found good agreement between VLDL-TG FCR obtained by MIDA and that obtained by any of the three glycerol approaches. Siler and collaborators (15) have recently compared parameters of TG production, during ethanol-stimulated lipogenesis, using the MIDA approach with either an infusion of [2-^{13}C]acetate or an infusion of [2-^{13}C]glycerol. Parameters of TG production were similar in the three subjects studied with these two approaches (15). However, it is important to note that their approach differs from ours as they used an infusion of glycerol (we used a bolus) and they obtained parameters of TG production by using the MIDA approach (we used the monoexponential decrease of VLDL-TG glycerol).

In conclusion, we have described a nonradioactive tracer method to measure VLDL-TG kinetics in humans. This method is a convenient, short duration (12 h) tool that permits the safe repeated measurement of VLDL-TG kinetics. Hence, it should be useful to evaluate VLDL-TG physiology and pathology and to assess the effects of therapeutic and other interventions that may impact on VLDL-TG metabolism.

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