Measuring VLDL-triglyceride turnover in humans using ex vivo-prepared VLDL tracer

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Abstract **There has been more interest in VLDL-triglyceride (TG) kinetics during the last decade. Unfortunately, robust measurement methods are elaborate and not readily available. Here, we describe a method using unique, ex vivo labeling of the fatty acid moiety of VLDL-TG followed by intravenous bolus infusion in the same person. We found that plasma disappearance of ex vivo-labeled VLDL-TG was comparable to that of in vivo-labeled VLDL-TG and that turnover rates can be safely estimated from the log linear decay of VLDL-TG specific activity. We found minor labeling of the plasma FFA (oleate) pool, which was largely attributable to coinfusion of free [^{14}C]triolein; VLDL-TG did not contribute substantially to the plasma FFA pool. The plasma decay curve of VLDL-TG was not affected by the presence of tracer in the FFA pool, provided that the data from 2 h after the VLDL tracer bolus infusion was used. The FFA contamination problem was circumvented by minor modification of the VLDL-TG tracer preparation. The approach we describe should expand the opportunity to study processes that cannot be assessed if the FFA precursor pool is labeled. This method for VLDL-TG tracer preparation can allow measurement of VLDL turnover, tissue uptake of VLDL-TG, and oxidation of VLDL-TG.—**Gormsen L. C., M. D. Jensen, and S. Nielsen. **Measuring VLDL-triglyceride turnover in humans using ex vivo-prepared VLDL tracer. *J. Lipid Res.* 2006.** 47: **99–106.**

Supplementary key words very low density lipoprotein • lipoproteins • free fatty acids

Our present understanding of human VLDL-triglyceride (TG) kinetics and metabolism has been limited by the lack of easily available and robust methods. Although the assessment of VLDL production using splanchnic balances is thought to be accurate (1, 2), this procedure requires blood sampling from the arterial bed as well as the hepatic and portal veins in combination with measurement of splanchnic blood flow, which is impractical in human experimental settings. Traditional turnover measurement, based on isotope dilution techniques using constant infusion of labeled VLDL-TGs (3), has not been widely used in human studies because of the lack of appropriate tracers and problems with tracer recycling. Consequently, a variety of alternative approaches have been used, as recently reviewed by Magkos and Sidossis (4). Among the most widely used approaches are labeling of the TG precursor pool with FFA (1–3), glycerol (8–11), or acetate (12), which results in in vivo incorporation of the tracer into the VLDL-TG complex. Frequent blood sampling and mathematical modeling of the data are needed to calculate the plasma VLDL-TG decay. These methods do not allow one to determine the metabolic fate of the fatty acid moiety of the TG molecule and are dependent upon the validity of the mathematical model for the given experimental setting. Another approach is to collect and isolate in vivo-labeled VLDL particles and reinfuse them into the same subject at a later time (13). However, using this approach, only a small amount of labeled VLDL-TG can been obtained for reinfusion; consequently, the specific activity (SA) of circulating VLDL-TG after reinjection has been sufficiently low that it is sometimes difficult to obtain accurate kinetic estimates. To better understand the relationship between adipose tissue lipolysis, hepatic lipoprotein synthesis, and the peripheral metabolism of VLDL-TG, it is necessary to use experimental models that accurately measure the turnover of both FFA and lipoprotein TG.

In the postabsorptive state, VLDL-TG production and clearance are in steady state; therefore, VLDL production can be determined from the disappearance rate of isotopically labeled VLDL. We have developed a new technique suitable for radiolabeling of VLDL-TG particles ex vivo. The technique involves the isolation and labeling of VLDL-lipoprotein particles with radiolabeled triolein by incorporating the tracer into the particles by gentle sonication. Moreover, by applying aseptic procedures, we have confirmed that the labeling process can be conducted under sterile conditions. The important question, how-

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ever, is whether ex vivo-labeled VLDL particles and in vivo-produced VLDLs have similar biological properties.

In these experiments, we *1*) compared the plasma decay of in vivo-labeled and ex vivo-labeled VLDL-TG; *2*) assessed the relationship between plasma concentrations of ex vivo-labeled VLDL-TG and the presence of the fatty acid label in FFA; *3*) determined the fate of TG when administered without being incorporated into VLDL; and *4*) revised the ex vivo labeling technique to eliminate the "free" TG.

MATERIALS AND METHODS

All protocols were approved by the Mayo Clinic Institutional Review Board (protocols 1 and 2a) or the Aarhus County Ethics Committee (protocols 2b, 3, and 4), and written informed consent was obtained before participation.

Subjects

Protocol 1. Four healthy men, aged 30–48 years (range), were selected so that two were normotriglyceridemic (1.25 and 1.58 mmol/l, respectively) and two were hypertriglyceridemic (4.30 and 5.25 mmol/l, respectively). All had been at a stable weight for at least 1 month before the study and consuming their customary diet for at least 1 week before the study.

Protocol 2a. Fourteen healthy subjects (five women and nine men), aged 23–44 years, were recruited among participants of an ongoing metabolic study. All had been weight-stable (±3 kg) for the last 3 months. In addition, all participants were provided weight-maintaining meals (50% carbohydrates, 30% fat, and 20% protein) in the Mayo Clinic General Clinical Research Center (GCRC) for 2 weeks before the studies and consumed all of their meals in the GCRC. The characteristics of these subjects are shown in **Table 1**.

Protocol 2b. Two healthy men, aged 27 and 29 years, with body mass index values of 22.4 and 24.8 kg/m^2 were recruited. All participants in both studies had normal physical examinations and laboratory evaluations. In addition, all were nonsmokers and abstained from strenuous exercise 48 h before the study day. None used any medications.

Protocol 3. Three healthy men, aged 21–25 years, were recruited. All had been weight-stable (±3 kg) for the last 3 months. All participants were in good health and had normal physical examinations and laboratory evaluations. In addition, all were nonsmokers and abstained from strenuous exercise 48 h before the study day.

TABLE 1. Subject characteristics (protocol 2)

Variable	Value
Number (women/men)	14 (5/9)
Age (years)	33.3 ± 1.8
Waist-to-hip ratio	0.90 ± 0.02
Body mass index (kg/m^2)	28.3 ± 1.5
Triglycerides (mmol/l)	1.84 ± 0.24
Total cholesterol (mmol/l)	5.0 ± 0.4
HDL cholesterol (mmol/l)	1.08 ± 0.06

Values shown are means ± SEM.

Protocol 4. Four healthy volunteers (two men and two women), aged 24–29 years, were recruited. Their characteristics were similar to those of the participants in protocol 2.

Supplies

L-[1-^{14}C]triolein, [9,10-^{3}H]triolein, and [1-^{14}C]palmitate (DuPont NEN Research Products and Perkin-Elmer Life Sciences, Boston, MA) were used in these studies. The tracers were assayed for radiochemical purity by measuring the radioactivity in the TG, non-TG, and palmitate fractions by HPLC.

In vivo VLDL-TG labeling: (during day 1 of protocol 1)

A bolus of 100 μCi of [1-^{14}C]palmitate was infused in an antecubital vein of each participant. After 90 min, 280 ml of blood was collected from the contralateral antecubital vein. The plasma was immediately separated, and VLDL lipoprotein was isolated by ultracentrifugation at 40,000 *g* for 18 h at 10°C in a 50.3 Ti rotor (Beckman Instruments, Palo Alto, CA) using aseptic technique for all procedures. The VLDL supernatant was removed using a modified Pasteur pipette, passed through a Millipore® filter (pore size diameter = 0.22 μm), and stored under sterile conditions at 5°C. A representative sample was tested to ensure apyrogenicity and sterility. After 1 week, the sample was resuspended in 10 ml of 0.9% saline and infused intravenously into the same subject.

Ex vivo VLDL-TG labeling

Two different procedures were used for this purpose. In procedure a, VLDL particles were isolated before tracer incorporation. In procedure b, labeling of VLDL was performed before isolation of the VLDL fraction (i.e., on whole plasma).

Procedure a. Under sterile conditions, a blood sample (20 or 30 ml) was obtained from each volunteer and the VLDL lipoprotein was separated as described above. The isolated VLDL-TG sample was then added to a sterile test tube containing dried [^{3}H]triolein (protocol 1) or [1-^{14}C]triolein (protocol 2) and sonicated in a water bath for 30 min (speed of 200/min) at 37°C. This procedure allows labeled TGs to become incorporated into the VLDL complex. Variable amounts of [^{3}H]triolein were used in these studies (3.5, 5, and 100 μCi in protocol 1; 20 μCi in protocol 2). The solution was then passed through a Millipore® filter (pore size diameter = 0.22 μm) and stored under sterile conditions at 5°C. Representative samples were tested to ensure apyrogenicity and sterility. After 1 week, the sample was resuspended in 10 ml of 0.9% saline and injected intravenously into the same subject. We conducted additional control experiments showing that such ex vivo-labeled VLDL-TG particles are indistinguishable from native VLDL with regard to electrophoretic properties, cholesterol-to-TG ratio, apolipoprotein B-100 (apoB-100) concentrations, and mobility on size-exclusion HPLC (14). When using this procedure, ~50–60% of the tracer was recovered and available for infusion.

Procedure b. A 40 ml blood sample was obtained under sterile conditions from each volunteer. Plasma was immediately separated and transferred to sterile test tubes containing 20 μCi of dried [1-^{14}C]triolein and sonicated in a water bath at 37°C for 6 h. The solution was then transferred to sterile tubes, and any possible chylomicrons and nonchylomicron TGs were isolated by a 30 min ultracentrifugation at 15,000 *g* using a 50.3 Ti rotor as described above. The supernatant was removed with a modified

Pasteur pipette. The infranatant was transferred to new sterile tubes, and the VLDL fraction was isolated by ultracentrifugation as described above. When using this procedure, ~10% of the tracer was recovered and available for infusion.

VLDL-TG purity and tracer incorporation

Both the in vivo- and ex vivo-labeled VLDL particles were assayed for purity and tracer incorporation by size-exclusion HPLC as described previously by Klein et al. (14) with minor modifications. A 200 μl sample mixed with 2 μl of Sudan Black (Fisher Scientific Co.) was injected on an HPLC system using two size-exclusion columns (Superose 6 HR 10/30; Pharmacia LKB Biotechnology, Uppsala, Sweden) using 0.15 M sodium chloride containing 0.005 M Tris, 0.0005 M EDTA, and 0.005% NaN_3 as running buffer and ultraviolet detection. The effluent peak of interest (VLDL-TG) was collected at 19–23 min in scintillation vials, dried down to a volume of 1–2 ml, resuspended in liquid scintillation cocktail, and counted by single-channel liquid scintillation counting. Control experiments in which the effluent TG peak was collected and reinjected onto the column, as well as measurement of SA in the sample and effluent, confirmed that >99% of the tracer that was put onto the column was associated with VLDL particles.

Oleate and VLDL-TG SA

Plasma oleate SA was measured by HPLC as described previously (15). To determine VLDL-TG SA, ~3 ml of each plasma sample was transferred into Optiseal tubes (Beckman Instruments), covered with a saline solution (d = 1,006 g/ml), and centrifuged (50.3 rotor; Beckman Instruments) for 18 h at 40,000 g and 10°C. The top layer, containing VLDL, was recovered using tube slicing (~1.5 ml), and the exact volume was recorded. A small proportion was then analyzed for TG content using a COBAS Integra 800, and plasma concentrations of VLDL-TG could then be calculated. The remaining VLDL-TG was transferred to a glass scintillation vial, 10 ml of scintillation liquid was added, and the sample was measured for ^{14}C activity using dual-channel counting.

Adipose tissue VLDL-TG uptake

Adipose tissue biopsies were obtained by needle liposuction technique under local anesthesia from abdominal and gluteal adipose tissues at 5 and 24 h after injection of the labeled VLDL-TG samples. Adipose tissue lipids were extracted using standard procedures, and the TG SA was measured as described by Marin, Rebuffe-Scrive, and Bjorntorp (16). The extracted lipid was accurately weighed and counted on a scintillation counter to <2% counting error. The adipose tissue TG SA (^{14}C dpm/mg lipid) was calculated.

Body composition

Total body fat and fat-free mass were measured 1 week before the study by dual-energy X-ray absorptiometry [Lunar Radiation Corp., Madison, WI (Mayo Clinic) and Hologic Discovery, Hologic, Inc., Bedford, MA (Aarhus Kommuneospital)].

Experimental design and data analysis

Subjects studied at the Mayo Clinic (protocols 1 and 2a) were admitted to the GCRC at 5 PM on the evening before the study and were given a standard mixed meal. After completion of the meal, participants fasted overnight. Subjects studied at Aarhus University Hospital (protocols 2b, 3, and 4) were admitted to the Department of Experimental Clinical Research at 10 PM on the evening before the study and investigated after a 10 h fast.

Protocol 1. The participants were studied on two separate days (i.e., day 1 and day 2) 1 week apart. On day 1, a 20 ml blood sample was obtained for ex vivo VLDL-TG labeling using procedure a, after which an intravenous catheter was inserted and 100 μCi of [1-^{14}C]palmitate was infused over 30 min. After 90 min, 280 ml of blood for VLDL lipoprotein isolation was collected from a contralateral antecubital vein as described above. The timing of the collection was guided from a pilot study of one healthy normotriglyceridemic man showing that peak SA of in vivo-labeled VLDL-TGs occurred ~90 min after the palmitate tracer infusion. One week later, an intravenous catheter was inserted into an antecubital vein for injection of in vivo- and ex vivo-labeled VLDL-TGs over 15 min using syringe pumps. Blood samples (10 ml) were collected at least every 0.5–2 h for 12–24 h for measurement of VLDL-TG SA. To avoid chylomicronemia, the subjects were allowed to drink noncaloric fluids and to eat nonfat foods with limited amounts of simple carbohydrates. Plasma VLDL-TGs were isolated by ultracentrifugation, and a small fraction was assayed for TG concentration. The remaining sample was analyzed for [^{14}C]VLDL-TG and [^{3}H]VLDL-TG activity using dual-channel liquid scintillation counting, and VLDL-TG SA was then calculated (dpm/mmol VLDL-TG). We previously conducted control experiments to confirm that the triolein tracer does not appear in the phospholipid fraction (17).

Protocol 2a. One week before the study day, a 30 ml venous blood sample was obtained under sterile conditions. VLDL-TGs were isolated from the sample using ultracentrifugation and subsequently labeled using 20 μCi of [1-^{14}C]triolein and procedure a. The labeled VLDL-TG sample was stored at 5°C until the study day. On the study day, the participant's own ex vivo-labeled VLDL sample was reinfused, and 10 ml blood samples were obtained after 30, 40, 50, and 60 min for determination of VLDL-TG SA and plasma free oleate SA as described above. The next morning, an adipose tissue biopsy was obtained by needle liposuction from abdominal subcutaneous adipose tissue and analyzed for tracer activity (16).

Protocol 2b. In the second study, the volunteers were also infused with ex vivo-labeled VLDL-TG particles that were collected, labeled, and administered as in protocol 2a. On the study day, 10 ml blood samples were collected for measurement of VLDL SA every hour for 5 h, and plasma samples from two volunteers were also assayed for plasma free oleate SA. At the end of the study, adipose tissue biopsies from abdominal subcutaneous and gluteal fat were obtained by needle liposuction from three subjects (one woman and two men) and analyzed for adipose tissue lipid SA.

Protocol 3. After an overnight fast, three healthy men received a bolus of 5 μCi of [1-^{14}C]triolein suspended in a 1,006 g/cm^3 NaCl solution intravenously. The 5 μCi dose was estimated to represent the maximum amount infused as free triolein in subjects infused with ex vivo [1-^{14}C]triolein-labeled (20 μCi) VLDL. Blood samples were collected every 30 min for measurement of VLDL-TG SA.

Protocol 4. After an overnight fast, four healthy volunteers (two men and two women) received a bolus infusion (2 μCi) of ex vivo-labeled VLDL-TGs labeled using procedure b. Blood

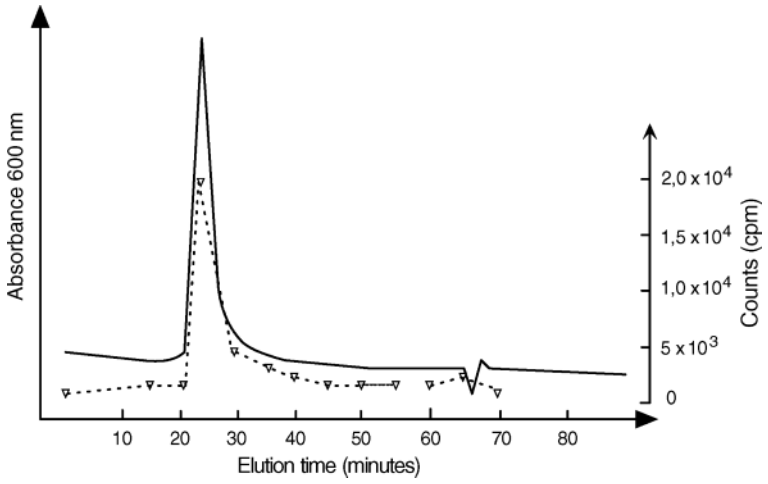

Fig. 1. Particle and radio chromatograms (size-exclusion) of isolated, ex vivo-labeled VLDL-triglyceride (TG) particles (solid and dashed lines, respectively).

samples were collected every 10 min for the first hour and every hour for 5 h for measurement of plasma VLDL and oleate SAs. Abdominal and gluteal adipose tissue biopsies were collected after 5 h.

Calculations and statistics

Data are reported as means ± SEM. VLDL-TG SA is expressed as dpm/μmol TG. Oleate SA is expressed as dpm/μmol oleate. The turnover of in vivo- and ex vivo-labeled VLDL-TGs was calculated from the monoexponential plasma decay curve of the individual isotopes. Slopes of individual curves were evaluated using linear regression analysis. Fractional catabolic rate (FCR) was calculated as $\ln(2)/T_{1/2}$, where $T_{1/2}$ indicates half-time. The fractional tracer uptake in adipose tissue was calculated as adipose tissue SA (dpm/g) × FM (g)/dose infused (dpm), where FM is the total fat mass as determined by dual-energy X-ray absorptiometry scanning.

RESULTS

Figure 1 shows representative size-exclusion VLDL particle and radio chromatograms of isolated, ex vivo-labeled VLDL particles (procedure a). The simultaneous elution of VLDL-TG lipoprotein fraction and tracer indicates that the triolein tracer was incorporated into the VLDL particles. Approximately 50% of the tracer used for sonication was recovered in VLDL for infusion.

Protocol 1: plasma disappearance of in vivo- and ex vivo-labeled VLDL-TGs

The SA of plasma [^{3}H]VLDL-TGs and [1-^{14}C]VLDL-TGs decayed in a similar manner (**Fig. 2**), indicating that there was no difference in the plasma turnover of these particles.

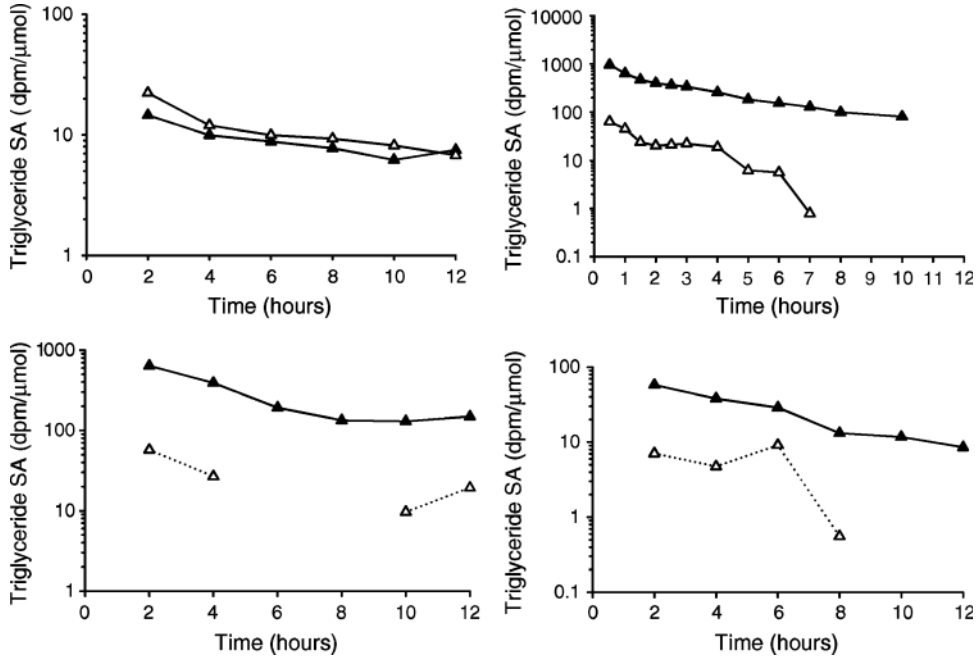

Fig. 2. Time course of plasma [^{3}H]VLDL-TG (closed triangles) and [1-^{14}C]VLDL-TG (open triangles) specific activity (SA; dpm/μmol TG) after bolus infusion of ex vivo-labeled VLDL-TGs at time zero in four healthy volunteers.

Despite using considerably less tracer, the [^{3}H]VLDL SA was notably greater than the [1-^{14}C]VLDL SA. In two of the participants, [1-^{14}C]VLDL SA decreased to near-background activity levels, resulting in less reliable SA measurements toward the end of the study period.

Protocols 2a and 2b: plasma VLDL-TG and oleate SA

Figure 3 shows the plasma [^{14}C-oleate]VLDL-TG SA disappearance from 30 to 60 min after bolus injection at time zero. Based on this short observation period, plasma $T_{1/2}$ was calculated to be 62.6 ± 3.7 min (range, 43.3–91.2 min) and FCR was 0.69 ± 0.04 pools/h (range, 0.46–0.96 pools/h). The figure also shows plasma free [^{14}C]oleate SA as well as the oleate-to-VLDL SA ratio (inset). Between 30 and 60 min, plasma oleate SA declined gradually in all volunteers, from 509 dpm/μmol (range, 110–1,291 dpm/μmol at 30 min) to 283 dpm/μmol (range, 0–823 dpm/μmol at 60 min), and remained above background in half of the volunteers. After 120 min, the VLDL-TG SA decayed in a log linear manner, whereas plasma oleate SA remained just slightly above background activity. During the same time, the relationship between plasma oleate SA and VLDL-TG SA was constant (Fig. 3, inset), with an average 10-fold (range, 8- to 11-fold) higher VLDL-TG SA compared with oleate SA. Because only two subjects were studied in protocol 2b, we chose not to calculate turnover rates.

We hypothesized that the greater ratio during the early decay phase indicated that the infusion of labeled VLDL included either some TG that had been incompletely incorporated into the VLDL complex during tracer preparation or free oleate, perhaps from residual lipase activity in plasma. Our rationale for this supposition is that the plasma free oleate SA originating from any normal, physiological escape of fatty acids in VLDL-TG (e.g., during lipoprotein lipase-mediated hydrolysis) should theoretically result in a constant (precursor-product) ratio. To assess whether the [^{14}C]triolein was undergoing ex vivo lipolysis (generating significant amounts of [^{14}C]oleate) during storage of the VLDL sample, we performed additional control studies to test the amount of free [^{14}C]oleate in the final labeled VLDL sample. Two healthy volunteers each provided two plasma samples that were subsequently labeled using methods A and B. Immediately after sonication and after 1 week of storage in the refrigerator, the labeled VLDL samples were analyzed for oleate concentration and SA by HPLC, TG concentration, and radioactivity in VLDL by size-exclusion HPLC. Free oleate was either undetectable or barely detectable (concentrations of 0–2 μmol/l), with no tendency toward an increase from day 0 to day 7. The radioactivity associated with the oleate fraction increased slightly in three samples and decreased slightly in one of the paired samples. The total dpm associated with the free oleate peak accounted for <0.01% of the label used to label the VLDL-TG, which is not enough to account for the appearance of the tracer in plasma free oleate. Thus, ex vivo lipolysis of [^{14}C]triolein cannot account for the changing ratio of VLDL-TG to free oleate SA depicted in Fig. 3. The other possible explanation for the results of protocol 2b is that some of the triolein tracer was incompletely associated with the VLDL particles and that rapid in vivo hydrolysis generated free oleate. Protocol 3 was conducted to assess the pattern of response to radiolabeled triolein infusion not associated with VLDL particles.

Protocol 3: VLDL SA after [1-^{14}C]triolein infusion

After infusion of free [1-^{14}C]triolein, immediate increases in plasma free oleate SA occurred (**Fig. 4**). We also observed ^{14}C appearing in plasma VLDL-TG after the infusion, with a peak in the VLDL SA at 2 h (Fig. 4). Thereafter, a log linear decline was observed. The peak VLDL-TG SA was less than the VLDL-TG SA observed after infusion of 1-^{14}C-labeled VLDL-TGs.

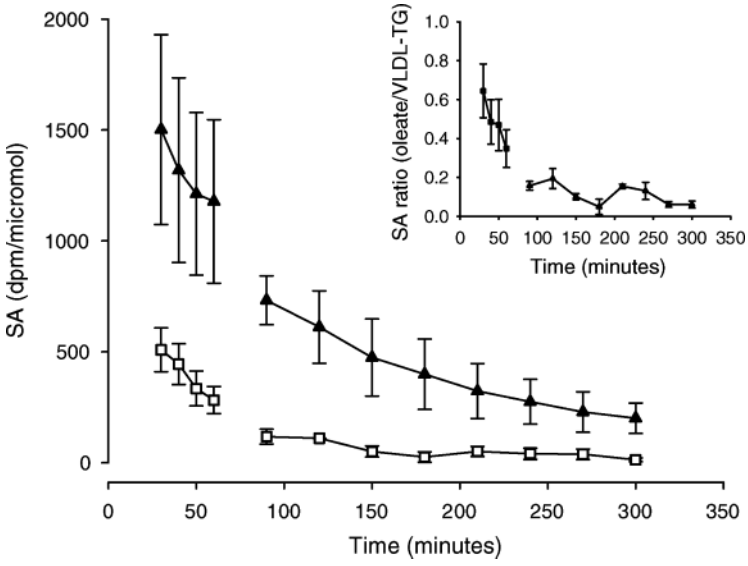

Fig. 3. Decay of plasma [1-^{14}C]VLDL-TGs (closed triangles) and oleate (open squares) SA (dpm/μmol TG and dpm/μmol oleate, respectively) after bolus injection of ex vivo-labeled VLDL-TGs at time zero. The inset shows the ratio relationship of plasma oleate SA to VLDL-TG SA during the observation period. Values shown are means ± SEM.

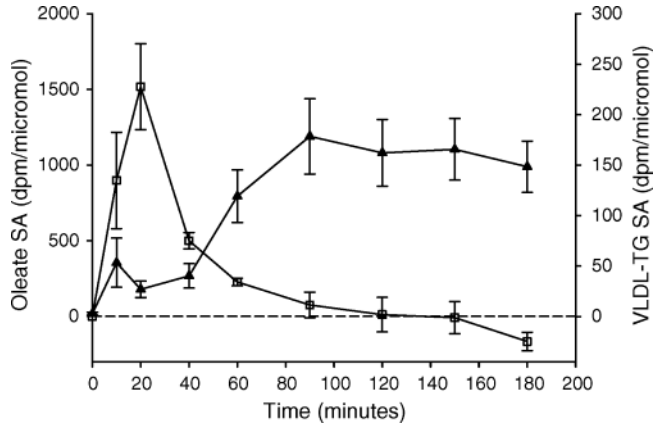

Fig. 4. SA (dpm/μmol) of oleate and VLDL-TG after infusion of 5 μCi of [1-^{14}C]triolein. Open squares, oleate SA (dpm/μmol fatty acid); closed triangles, VLDL-TG SA (dpm/μmol TG). Values shown are means ± SEM.

Protocol 4: VLDL-TG and oleate SA (modified tracer preparation)

Figure 5 shows the plasma oleate SA and VLDL-TG SA of volunteers infused with VLDL particles labeled ex vivo according to procedure b. As can be seen, this approach resulted in the nominal appearance of tracer in plasma free oleate and a log linear VLDL-TG SA decay from as early as 20 min after the infusion.

Adipose tissue tracer uptake (protocols 2 and 4)

After infusion of ex vivo-labeled VLDL-TG particles, 1-^{14}C tracer uptake was demonstrated in adipose tissue biopsies and was greater after 24 h compared with 5 h. Fractional uptake [adipose tissue SA (dpm/g) × FM (g)/dose infused (dpm)] was calculated to 18.6 ± 5.0% (range, 5.9–40.7%) after 24 h. In fat biopsies obtained after 5 h, fractional uptake in adipose tissue of the two men and one woman was 6.6, 8.6, and 13.6%, respectively, with no apparent difference between abdominal and gluteal fat uptake.

DISCUSSION

The purpose of the present studies was to evaluate the suitability of ex vivo labeling of isolated VLDL-TGs in assessing VLDL-TG kinetics. The participants' own VLDL particles were collected, purified, and radiolabeled, then reinfused. The disappearance of in vivo- and ex vivo-labeled VLDL particles from plasma was similar in each participant, indicating that ex vivo-labeled particles are cleared from plasma by the same mechanism(s) (e.g., lipoprotein lipase) as in vivo-labeled TGs particles. Moreover, we found sufficient uptake of the tracer in adipose tissue biopsies to allow quantitative assessment of regional fractional VLDL-TG uptake. Although this procedure is associated with the potential risk of administering pyrogens or bacterially contaminated materials, we have demonstrated that we can carry out these procedures in our laboratory under sterile conditions.

Our studies support the notion that ex vivo-labeled VLDL particles are indistinguishable from native VLDL with regard to electrophoretic properties, cholesterol-to-TG ratio, apoB-100 concentration, and mobility on size-exclusion HPLC (14). VLDL particles are complex structures consisting of a core region containing hydrophobic lipids, principally TG and cholesteryl esters. Surrounding the core is a spherical phospholipid monolayer in which apoB-100 is located. Whereas the turnover of the apoB-100 molecule reflects the VLDL particle turnover (7, 18), individual TG molecules may be attached to the VLDL complex in a temporary manner. The turnover of apoB-

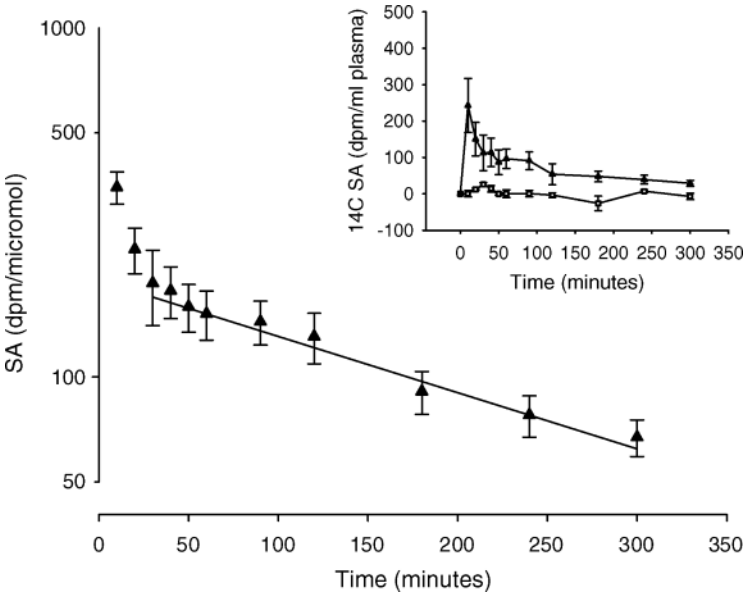

Fig. 5. Plasma VLDL-TGs (closed triangles) and oleate (open squares) SA of volunteers infused with VLDL particles labeled ex vivo according to procedure b (see text). This approach resulted in nominal plasma oleate SA (see inset) and a log linear VLDL-TG SA decay. Values shown are means ± SEM.

100 and VLDL-TGs, therefore, may not be identical. In fact, VLDL-TG synthesis has been shown to be dissociated from apoB synthesis during the stimulation of VLDL particle production after a high-carbohydrate diet (19, 20).

An inherent problem in tracer studies relates to the possibility of tracer recycling, which in most cases leads to the underestimation of tracer turnover. In these studies, we used two different procedures to generate our VLDL tracer. When using procedure a, it seems that unbound [1-^{14}C]triolein was inadvertently coinfused with the labeled VLDL-TG tracer. Such free triolein molecules appear to be rapidly hydrolyzed, resulting in free [^{14}C]oleate, which then appears to be taken up by the liver and incorporated into VLDL particles. When using procedure a to label VLDL-TG, we found that plasma free oleate SA increased after 30 min but declined rapidly within the first 120 min after infusion of VLDL. After this time point, there was no evidence of ongoing generation of radiolabeled free oleate because there was a constant relationship between oleate SA and TG SA from 120 to 300 min. We believe that intravascular hydrolysis of unbound [1-^{14}C]triolein associated with ex vivo-labeled lipoprotein particles from procedure a resulted in the appearance of the labeled free oleate. Infusion of unbound [1-^{14}C]triolein reproduced this finding, and we could eliminate this phenomenon using procedure b to label VLDL-TG. Our data suggest that the postabsorptive contribution of plasma VLDL-TG to the plasma FFA pool is small. That said, it appears that a minor proportion of the fatty acids from plasma VLDL-TG escape into the plasma FFA pool as a result of lipoprotein lipase activity.

These appear to be the first studies to examine the fate of an infusion of free [1-^{14}C]triolein. We found that plasma VLDL-TG SA increased within 30 after the infusion (similar to that seen after a tracer infusion of FFA) and peaked after ~2–3 h. Thereafter, a log linear decline in VLDL-TG SA was observed. The similar decay of in vivo- and ex vivo-labeled particles after 2 h (Fig. 2) as well as the constant precursor-product ratio indicate that the contribution of free oleate to VLDL SA as well as tracer recycling is nominal after 2 h. Together, these findings indicate that when using procedure a, turnover calculation should be performed after 2 h to avoid quantitatively important tracer recycling. If procedure a is used, it would be important to reisolate the VLDL particles to avoid coinfusion of free triolein tracer. Alternatively, this problem can be circumvented by using procedure b. This approach did not result in significant free oleate appearing in plasma after VLDL infusion, making it feasible for many experimental settings. However, labeling of isolated VLDL-TGs (procedure a) resulted in greater adipose tissue uptake of tracer, which reflects the fact that more tracer was infused compared with procedure b. Because free triolein is coinfused when using procedure a, only relative adipose tissue VLDL-TG uptake (e.g., upper body subcutaneous vs. lower body adipose tissue) can be estimated with some confidence, whereas total fractional adipose tissue uptake cannot. Again, this drawback can be circumvented by using procedure b.

This study is the first to use uniquely labeled and isolated VLDL-TGs in humans without prior labeling of the TG precursor pool (FFA, glycerol, or acetate), the limitations of which have been discussed previously. We found VLDL-TG turnover rates to be compatible with previously reported estimates (4). However, because our volunteers constituted a heterogeneous group with respect to gender and obesity phenotype, no conclusions regarding the actual turnover rates (VLDL production and FCR) or adipose tissue tracer uptake can be made. A distinct advantage of ex vivo labeling is the improved efficiency of developing a VLDL tracer. In vivo labeling of TG-associated FFAs involves administering radiolabeled FFAs to a volunteer and collecting the VLDL particles several hours later. Only 1–2% of the tracer is actually recovered for later reinfusion. In contrast, with ex vivo labeling we found ~10–20% recovery of tracer in the VLDL fraction on HPLC. Moreover, this method offers the ability to measure VLDL-TG kinetics with a reduced exposure to radioisotopes. An early approach to study VLDL kinetics used sonication procedures to ex vivo incorporate radiolabeled cholesteryl linoleyl ether into rat lipoprotein particles (21). This method was reported to give similar VLDL turnover values compared with in vivo-labeled VLDL. Eaton, Berman, and Steinberg (13) performed studies in humans using in vitro autologous labeling of lipoproteins with [^{14}C]triolein and reinjection of the labeled sample. However, no attempt was made to separate individual lipoproteins before performing the labeling procedure; in fact, the authors reported that radiolabeled TG was found in all lipoprotein fractions before injection of the processed sample. In a recent elegant study, Sidossis and coworkers (3) used in vivo labeling of VLDL-TGs using [U-$^{13}C_3$]glycerol, plasmapheresis, and isolation of ^{13}C-labeled VLDL-TGs followed by traditional isotope dilution technique (primed constant infusion and steady-state equations). Such studies require the availability of mass spectrometry facilities. However, the choice of tracer precludes this approach from evaluating peripheral tissue uptake and tissue metabolism.

On the basis of the SA of the [1-^{14}C]triolein we used for these studies and the resulting VLDL-TG SA, we estimate that the tracer adds only ~0.5% to the TGs in VLDL particles. Thus, it may be difficult to use stable isotope techniques to reproduce this approach, although ultra-low doses of U-^{13}C-labeled fatty acids and gas chromatography/combustion/isotope ratio mass spectrometry can be used to measure FFA turnover (22), and it may be possible to create TGs using uniformly labeled fatty acids for VLDL-TG labeling.

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