A novel method for measuring intestinal and hepatic triacylglycerol kinetics

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Sun F, Stolinski M, Shojae-Moradie F, Lou S, Ma Y, Hovorka R, Umpleby AM. A novel method for measuring intestinal and hepatic triacylglycerol kinetics. Am J Physiol Endocrinol Metab 305:E1041–E1047, 2013. First published April 16, 2013; doi:10.1152/ajpendo.00105.2013.—This study aimed to J develop a method that completely separated hepatic (VLDL1, VLDL2) and intestinal [chylomicron (CM)] lipoproteins and 2 use the method to measure triacylglycerol (TAG) kinetics in these lipoproteins in the fed and fasting state in healthy subjects, using intravenous [2H5]glycerol as the tracer. An immunoaffinity method that completely separated hepatic and intestinal particles using sequential binding to three antibodies to apolipoprotein B-100 (apoB-100) was established and validated. Six healthy volunteers were studied in a fasted and continuous feeding study (study 1). Five additional healthy volunteers were studied in a continuous feeding study that included an oral [13C3]glycerol tripalmitin tracer (study 2). In both studies, an intravenous bolus of [2H5]glycerol was administered to label TAG in hepatic and intestinal lipoproteins. In both feeding studies there was sufficient incorporation of the [2H5]glycerol tracer into the exogenous lipoproteins to enable isotopic enrichment to be measured. In study 2, the oral tracer enrichment in VLDL1 was <5% of CM enrichment 150 min after tracer administration, demonstrating negligible contamination of VLDL1 with apoB-48. Western blotting showed no detectable apoB-100 in CMs. VLDL1 and VLDL2 TAG fractional catabolic rate (FCR) did not differ between feeding and fasting (study 1). There was no difference between CM and VLDL1 TAG FCR in both fed studies. In fed study 2, 47% of the total TAG production rate (CM + VLDL1) was from CM. This methodology may be a useful tool for understanding the abnormalities in postprandial TAG kinetics in metabolic syndrome and type 2 diabetes.

very-low-density lipoprotein; chylomicron; stable isotopes; immunoaffinity; postprandial

There is considerable evidence that increased fasting plasma triacylglycerol (TAG) levels are an independent risk factor for cardiovascular disease (4) and play a role in the development of atherosclerosis (17). However, postprandial hypertriglyceridemia may be a more important risk factor for atherosclerosis than fasting hypertriglyceridemia (37), especially on a Western diet, since TAG levels are elevated for most of the day due to the frequent consumption of food (35).

Hypertriglyceridemia is due to the presence of excess TAG-rich lipoproteins (TRL), which consist of very low-density lipoproteins (VLDL) synthesized by the liver and chylomicrons synthesized in the small intestine in response to an intake of dietary fat. Measurement of VLDL flux using isotopic tracers has provided an invaluable insight into the abnormalities of VLDL metabolism in various diseases (3, 10). During fasting, VLDL metabolism can be measured with a fatty acid (18) or glycerol tracer to measure TAG kinetics (24) and in fasting and feeding by labeling the main protein component apolipoprotein B (apoB)-100 (10) with an amino acid tracer to measure particle kinetics. In the postprandial period, measurement of TAG flux becomes more difficult because feeding is a nonsteady state. To enable quantitation of postprandial kinetics using isotopic tracers, a steady state is required, and a number of research groups have adopted a frequent feeding protocol to achieve this. Since on a Western diet people have elevated TAGs throughout the day (35), this is a reasonable approximation to the normal physiological state.

There are technical difficulties in separating hepatic and intestinal TAGs that overlap in particle density. To study postprandial TAG kinetics, it is important to be able to separate these two populations of particles. Since hepatic TRL contain apoB-100 and intestinal TRL contain apoB-48, which is 48% of the protein sequence of apoB-100, immunoaffinity chromatography with antibodies specific for the epitopes close to the COOH-terminal of apoB-100 (8, 15) have been used to separate the different populations of particles. Although a pure population of apoB-100 lipoproteins was obtained using these protocols, the apoB-48 population was reported to be contaminated with apoB-100. This has precluded an understanding of TAG metabolism in the intestinal lipoprotein pathway. Labeling apoB-48 rather than TAG has been used in several studies (20, 36) to give a measurement of exogenous lipoprotein particle kinetics rather than the kinetics of the TAG substrate within the particle.

The administration of an intravenous bolus of [2H5]glycerol is well validated for the measurement of VLDL TAG kinetics in the fasting state (12, 27). It is not known whether it can be used to study postprandial hepatic or intestinal TAG kinetics. We hypothesized that intravenously administered [2H5]glycerol could cross the basolateral membrane of the enterocyte since the aquaglyceroporin AQP3, which transports water and glycerol, has been identified in the basolateral membrane of the enterocytes in the human small intestine (7). Within the enterocyte the tracer could mix with free glycerol, which would be incorporated into TAG. It has been shown that 20–30% of TAG synthesized in the enterocyte is synthesized by the glycerol phosphate pathway (23). The small intestine is an insulin-sensitive gluconeogenic organ, and glycerol has been shown to be converted to glycerol 3-phosphate by glycerol kinase and then metabolized to glucose (9) or incorporated into triglyceride (31).

In this study, an immunoaffinity method that completely separated hepatic and intestinal lipoproteins was developed and validated. Using a 2-h frequent feeding protocol, it was shown...
that an intravenous glycerol tracer can be used to measure both hepatic and intestinal TAG kinetics.

**MATERIALS AND METHODS**

**Subjects**

This study was approved by the West Kent National Health Service Research Ethics Committee and University of Surrey Ethics Committee. Written, informed consent was provided by all participants prior to inclusion in the study. Four healthy volunteers (1 male and 3 females, aged 37.8 ± 5.8 yr; means ± SE) were recruited for measuring TAG binding capacity and validating the immunoaffinity methodology. Six healthy volunteers (2 males and 4 females, 36.8 ± 3.9 yr) with a BMI of 24.4 ± 1.1 were recruited for the measurement of hepatic and intestinal TAG kinetics in the fed and fasting state (study 1). Five healthy males (56.6 ± 3.0 yr, BMI 23.4 ± 0.3) were recruited for further validation of the immunoaffinity method for the measurement of hepatic and intestinal TAG kinetics in the fed state only (study 2). They had no history of hepatic, cardiac, renal, or endocrine dysfunction and were not taking medications known to alter plasma lipid levels.

**Experimental Design**

**Validation study.** Subjects were studied after an overnight fast. A blood sample was taken after 3 and 4 h after a meal (552 kcal, 8.7 g of protein, 38.5 g of carbohydrate, and 40.7 g of fat) was consumed, and Svedberg flotation rate (Sf) > 60 and Sf 20–60 lipoproteins were isolated.

**Study 1.** Subjects underwent a 7-h-fasted study and a 13-h-fed study in random order, with ≥2 wk between each study (Fig. 1). They had the same meal the evening before both studies, which was consumed before 10 PM, and then they fasted overnight. In both studies, an indwelling cannula was inserted into an antecubital vein for blood sampling. In the fasted study, after a baseline sample was taken, a bolus of 750 mg of [2H5]glycerol dissolved in saline [30 mg/ml, 2.5 ml/vial (Cambridge Isotopes), prepared aseptically in Guy’s and St. Thomas’ Hospital Trust Pharmacy] was injected into the contralateral antecubital vein. Blood samples were taken at intervals over 420 min for the measurement of Sf > 60 and Sf 20–60 lipoproteins enrichment and concentration and plasma glycerol enrichment and concentration.

In the fed study, subjects were fed a high-fat (88% energy), low-carbohydrate (11% energy), and low-protein (1% energy) liquid meal, 486 kcal/meal, every 2 h between −240 and 420 min. The meal, composed of 15 g of glucose, 40 ml of double cream, 15 ml of olive oil, and 15 ml of sunflower oil, was prepared as an emulsion. Each unit meal was prepared immediately prior to consumption and consumed within 5 min by the subject. A bolus of 750 mg [2H5]glycerol was administered after 4 h of feeding (0 min). Blood samples were taken for the measurement of Sf > 60 and Sf 20–60 lipoprotein TAG kinetics and plasma glycerol enrichment at −240 min and after administration of tracer at the same time as in the fasted study (Fig. 1).

**Study 2.** Subjects underwent a fed study, as described above, and in addition to the intravenous [2H5]glycerol were given an oral bolus of tripalmitate ([13C3]glyceryl, 10 mg/kg body wt; Cambridge Isotopes) emulsified with the third meal (0 min; Fig. 1).

**Lipoprotein Separation**

If the Sf > 60 and SF 20–60 lipoproteins were isolated by sequential flotation ultracentrifugation (14, 34) in a fixed-angle rotor 50.4Ti (Beckman), using a LE80-K ultracentrifuge (Beckman Coulter Optima).

**Isolation of Endogenous and Exogenous Lipoproteins**

Monoclonal antibodies to apoB-100, 4G3, 5E11, and Bso16 (75 µg, 1 mg/ml; Heart Institute, University of Ottawa, Ottawa, ON, Canada) were coupled individually to 120, 80, and 180 µg of protein G-Sepharose 4 Fast Flow (Amersham) in 20 mM sodium phosphate buffer, pH 7.0 (coupling buffer), in separate vials by rotating gently overnight. After centrifugation, the supernatant was removed. The protein G-antibody matrix was washed twice with coupling buffer and then packed into a glass vial and used immediately. After ultracentrifugation, 0.5 ml of lipoprotein fraction samples from the fed studies were loaded immediately onto the 4G3 matrix and incubated overnight. The next day, the 4G3 matrix with loaded lipoprotein samples was centrifuged, the supernatant was removed, and the bound fraction washed twice with coupling buffer and stored at 4°C. The supernatants after each centrifugation were combined and loaded on to the 5E11 matrix, incubated at 4°C overnight, and then centrifuged to separate the unbound and bound lipoproteins. The bound fraction in the slurry was washed twice, as described before. The supernatants after centrifugation were combined and loaded onto the Bso16 matrix.
and incubated at 4°C overnight. The unbound fraction (apoB-48 containing lipoprotein) in the final Bsol16 matrix separation was separated by centrifugation. The bound lipoproteins in the slurry after each separation on the 4G3, 5E11, and Bsol16 matrices (apoB-100-containing lipoproteins) were combined, centrifuged to remove the supernatant, and washed twice with buffer, and the supernatants were removed after centrifugation. This method was compared with a one-step separation using a mixture of the same amount of three different antibody matrixes and with sequential separation with two antibodies, 4G3 and 5E11. Optimal binding capacity of the antibody matrix was determined (data not shown). For optimum binding, 1-ml samples containing 1 mmol/l of TAG and 0.2 mg of each antibody (4G3, 5E11, and Bsol16) coupled to protein G-Sepharose were used. Samples with a higher concentration of TAG were diluted before loading on to the antibody matrix.

**Efficiency of Separation of apoB-100 and apoB-48 Particles**

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with protein silver staining (Bio-Rad) was used to determine whether the bound fractions were contaminated with apoB-48 and that the unbound fractions were not contaminated with apoB-100. A purified low-density lipoprotein (LDL) sample was used to locate the position of apoB-100 bands on SDS-PAGE. The position of apoB-48 was verified by Western blot using a specific antibody against human apoB-48 (32). Western blot was also used to verify the absence of apoB-100 in the unbound fractions. A monoclonal antibody against apoB-100 (5E11; University of Ottawa Heart Institute, Ottawa, ON, Canada) and apoB-48 (J-151; University of Ottawa Heart Institute, Ottawa, ON, Canada) was used as primary antibodies in Western blotting. A donkey anti-mouse antibody was used as the second antibody (Bio-Rad). A colorimetric kit (Alkaline Phosphatase Conjugate Substrate Kit; Bio-Rad) was used to visualize the bands.

SDS-PAGE and scanning densitometry (GS 800 Calibrated Densitometer; Bio-Rad) was used to quantify apoB-100 concentration (20) in the total Sf > 60 fraction and in the bound fractions, isolated by immunoaffinity, to calculate the recovery of apoB-100. Quantity One 1-D Analysis Software (version 4.6.3; Bio-Rad) was used to calibrate the protein concentration. The recovery of apoB-100 in the unbound fractions was also verified by Western blotting (Fig. 2B).

**Isotopic Enrichment Determination**

TAG in the lipoprotein fractions isolated by ultracentrifugation (fasted) or by immunoaffinity chromatography (fed) was extracted in chloroform-methanol (2:1, vol%)), separated by thin-layer chromatography with the mobile phase hexane-diethyl ether-acetic acid (70:30:2, vol%), and hydrolyzed with 3% HCl in methanol (vol%) at 50°C overnight. The separated lipid bands were visualized under iodine vapour. Glycerol was then purified by ion exchange chromatography and concentrated by freeze-drying overnight (1). For analysis of plasma glycerol, 0.5-ml samples were deproteinized with 1 ml of 3.5% (wt/vol%) sulfosalicylic acid and purified by ion exchange chromatography (1). Freeze-dried glycerol was derivatized to glycerol triacetate (1), and enrichment was measured by gas chromatography-mass spectrometry (Agilent 5973) in PCI mode, with methane as the reagent gas. Ions were monitored at m/z 159 and m/z 164 (M + 5).

The 13C/12C isotope ratio determination of glycerol in Sf > 60 unbound (CM) and Sf > 60 bound (VLDL1) in study 2 was performed with a Trace GC Ultra, with an auto sampler AS3000 coupled to an isotope ratio mass spectrometer Delta Plus XP (Thermo Electron) via an oxidation reactor, reactor temperature of 960°C, and a combustion interface III (Thermo Electron, Bremen, Germany).

**Kinetic Analysis**

The enrichment of the unbound Sf 20–60 was very low and near the limit of detection of the gas chromatography-mass spectrometry so that it could not be used for modeling. The fractional catabolic rates (FCR) of VLDL1 (Sf > 60 bound) and VLDL2 (Sf 20–60 bound) TAG in the fasting and fed state and CM TAG (Sf > 60 unbound) in the fed state were calculated using two different compartment models, one to describe VLDL TAG kinetics and one to describe CM TAG kinetics. Both models represented the kinetics of the tracer-to-tracee ratio (TTR) profiles that change as labeled glycerol is removed from plasma and incorporated into the TAG fractions. The incorporation of glycerol into VLDL TAG in the liver and into CM TAG in the enterocyte was subject to a delay. The VLDL model included a compartment for VLDL1 TAG and a compartment for VLDL2 TAG, with an input into both compartments from the glycerol precursor pool, a loss from each compartment, and a transfer from the VLDL1 TAG compartment to the VLDL2 TAG compartment. A single-pool model was used to describe the kinetics of CM TAG in the fed studies. In a steady state, the fractional secretion rate is equal to FCR. The production rate was calculated from the product of the fractional secretion rate and the TAG pool size. TAG pool size was calculated from the TAG concentration of the bound and unbound fractions multiplied by plasma volume. Plasma volume was calculated as described by Pearson et al. (29). [3H]-glycerol and [13C3]-glycerol tracer concentration corrected for dose of tracer was calculated as 

\[
\frac{3H}{13C} \text{glycerol or } \frac{13C}{12C} \text{glycerol} \times \text{TAG fraction concentration (µmol/l)/tracer dose (µmol)}.
\]

**Statistical Analysis**

Data were analysed using SPSS version 12.0.2 (SPSS, Chicago, IL). Nonparametric data, e.g., the FCRs, were logarithmically transformed before statistical analysis. Analysis of TAG concentrations during postprandial steady state was by repeated-measures ANOVA. Comparison of measurements in the fasted and fed studies was by paired two-tailed t-test. Comparison of measurements in fed study 1 and fed study 2 was by unpaired two-tailed t-test.

**RESULTS**

**Validation of Immunoaffinity Technique to Isolate Endogenous From Exogenous Lipoproteins**

The immunoaffinity technique using three-monoclonal antibodies (4G3, 5E11, and Bsol16) was able to completely separate apoB-100- from apoB-48-containing lipoproteins, as demonstrated by two independent methods. This was first shown by the absence of apoB-48 in the bound fractions and the absence of B100 in the unbound fractions by SDS-PAGE in the Sf > 60 and Sf 20–60 fractions (Fig. 2A). The absence of B100 in the unbound fractions was also verified by Western blotting (Fig. 2B). The use of three antibodies achieved a better separation of apoB-100- and apoB-48-containing lipoproteins than using two antibodies, and sequential separation achieved a complete separation of these two populations compared with a one-step separation using a mixture of the same amount of three different antibody matrices (data not shown). No TAG could be detected in the unbound fraction of fasting Sf > 60 or Sf 20–60 samples, demonstrating that there was no incomplete
binding or breakdown of the apoB-100 particles using the immunoaffinity technique (data not shown). Further evidence to demonstrate the complete separation of endogenous from exogenous particles was obtained in study 2 after administration of an oral $[13C_3]$glycerol triplamitin tracer and separation of the Sf > 60 fraction by the immunoaffinity technique. The enrichment of TAG in Sf > 60 showed that the majority of enrichment was in the unbound fraction (CM TAG), with very low enrichment in the bound fraction (VLDL1 TAG). $[13C_3]$glycerol tracer enrichment in VLDL1 was < 5% of CM enrichment 150 min after tracer administration, demonstrating the effectiveness of the immunoaffinity method at separating apoB-48 and apoB-100 particles (Fig. 2C). There was a small rise in enrichment of VLDL1 with $[13C_3]$glycerol during the second half of the study.

TAG Kinetics in the Fasted and Fed State (Study 1)

Fasting plasma TAG was 0.74 ± 0.05 mmol/l. In the fed study, the mean steady-state TAG concentration was 1.61 ± 0.03 mmol/l. This was not significantly different between 0 and 420 min. $[^{3}H_3]$glycerol enrichment of VLDL1 (Sf > 60) and VLDL2 (Sf 20–60) TAG in the fasted and fed studies is shown in Fig. 3. A and B. $[^{3}H_3]$glycerol enrichment of CM TAG (Sf > 60) in the fed study is shown in Fig. 4A. In the fasted and fed study, VLDL1 TAG FCR and VLDL2 TAG FCR were not different from each other or between studies (Fig. 5A).

In the fed study, the FCRs for CM TAG (25.4 ± 4.2 pools/day), VLDL1 TAG (19.6 ± 3.7 pools/day), and VLDL2 TAG (22.0 ± 4.3 pools/day) were not significantly different (Fig. 5A). In these studies TAG concentration in the bound and unbound fractions was not measured, so production rate could not be calculated.

TAG Kinetics in the Fed State (Study 2)

Fasting plasma TAG was 0.94 ± 0.12 mmol/l. In the fed state, the mean steady-state TAG concentration was 2.04 ± 0.27 mmol/l. This was not significantly different between 0 and 420 min. The TAG pool size in the CM, VLDL1, and VLDL2 fractions separated by the immunoaffinity technique was 1,186 ± 248, 1,631 ± 189, and 183 ± 22 mg, respectively. The tracer concentration for CM TAG labeled with $[^{3}H_3]$glycerol and $[13C_3]$glycerol, corrected for the dose of tracer administered, was similar, as shown in Fig. 4B. There was a 45-min delay in the appearance of the $[13C_3]$glycerol TAG. The peak in $[^{3}H_3]$glycerol TAG concentration was at 90 min, and the peak in $[13C_3]$glycerol TAG was between 180 and 240 min.

CM TAG FCR was not significantly different from VLDL1 TAG FCR (14.66 ± 2.08 vs. 10.55 ± 1.16 pools/day; Fig. 5B).
CM TAG FCR and VLDL1 TAG FCR were higher in fed study 1 than study 2 (P = 0.04, P = 0.03). CM and VLDL1 TAG production rates were similar to each other in study 2 (Fig. 5C).

**DISCUSSION**

In this study, a novel immunoaffinity technique, which separated hepatic and intestinal lipoprotein fractions while keeping the lipoprotein particle intact, was developed and validated. Using this method, we showed that with an intravenous bolus of [2H5]glycerol there was sufficient incorporation of the intravenous glycerol tracer into the intestinal lipoproteins to enable isotopic enrichment to be measured. With a continuous feeding protocol, TAG kinetics were measured within hepatic and intestinal lipoprotein particles in the fed state. Using this technique, we showed that the FCR of VLDL1 and VLDL2 in the fasted state did not differ from the equivalent fraction in a fed state in healthy subjects. The study also measured VLDL and CM TAG production rate for the first time in a separate study during a continuous feeding protocol and showed that 47% of total TAG production in the Sf > 60 fraction was from CM.

Previously, 4G3 and 5E11 antibodies to apoB-100 have been used in immunoaffinity chromatography to separate apoB-100 from apoB-48-containing lipoproteins (8, 15). Marcel et al. (25) found that as apoB-containing lipoproteins become smaller the conformation of specific regions of the apoB protein are modified. In the receptor-binding domain the conformation of the epitope recognized by 4G3, which is mapped between residues 2,980 and 3,080, remains constant, whereas that of 5E11, which recognizes the epitopes between 3,441 and 3,568, changes progressively. Therefore, a combination of 5E11 and 4G3 antibodies has generally been used to separate apoB-100 from apoB-48. However, whereas this method isolates a pure apoB-100 lipoprotein fraction, the apoB-48 fraction is contaminated with B100 to varying degrees (8, 15). Unlike previous studies that have used CNBr-activated sepharose, this study used protein G-Sepharose. Whereas CNBr-activated sepharose 4B interacts with amino groups, protein G-Sepharose binds to the Fc region of IgG, and therefore, the Fab region of the antibody is always available for antigen binding (1). This reduces the quantity of antibodies required since binding is more efficient. However, unlike CNBr-activated Sepharose, the antibodies cannot be reused. In this study, we found that by using the antibody Bsol16 (33), which recognizes the epitopes between 4,154 and 4,189, with sequential separation with each antibody, and using protein G as the matrix, there was a complete separation of endogenous and exogenous lipoproteins, as shown by SDS PAGE. Further validation with an orally administered tracer tripalmitate ([13C3]glyceryl), which...
would only be incorporated into chylomicrons, also showed that enrichment of \[^{14}C\]glycerol in the apoB-100 SF > 60-
containing lipoproteins was very low, indicating little contami-
nation with SF > 60 apoB-48 fractions. The small rise in
enrichment between 150 and 420 min is consistent with recy-
cling of CM TAG to VLDL1 TAG, as shown previously (16).

The isolation of intestinal lipoprotein TAG has long been an
issue that has restrained the measurement of its kinetics. The
labeling of apoB-48 rather than TAG has been used in several
studies (20, 36). This can be separated from apoB-100 by SDS
PAGE and can give a measure of exogenous lipoprotein
particle kinetics rather than the kinetics of the TAG substrate
within the particle. Investigators have also previously used
retinyl esters as tracers to measure chylomicron metabolism.
The use of retinyl esters during the initial postprandial period
may give some useful information about the metabolism of
intestinally derived particles (19); however, Krasinski et al.
(22) found that this method was not ideal for accurate mea-
surement in a longer period of study (>3–6 h postprandially).

In the current study, the development of a method that sepa-
rated intestinal lipoproteins made it possible to measure the
intestinal TAG kinetics in the fed state using an isotopic tracer
that labeled TAG.

It is well established that VLDL1 AND VLDL2 TAG kinetics can be measured in vivo using \[^{3}H\]glycerol (2, 5, 30)
in the fasted state. VLDL1 and VLDL2 TAG FCR in the
fasting study (study 1) were higher than in a previous study that
measured the kinetics of these fractions in healthy subjects (2).
There was a wide range of FCRs in both studies, with an
overlap in the ranges. The difference between the studies may
due to differences in the fasting TAG concentration, which
was higher (range 0.99–2.59 mmol/l) than in the current study
(range 0.64–0.98 mmol/l).

This study investigated whether administration of intrave-
nously labeled glycerol could be used to measure VLDL1 and
VLDL2 TAG kinetics in a continuous feeding study and
whether there would be sufficient incorporation of the tracer
into intestinal derived lipoproteins, i.e., chylomicrons to mea-
sure their kinetics. Although it has been shown that the major-
ity of dietary TAG is hydrolyzed into 2-monoglyceride and
fatty acid and then resynthesized to TAG via the monoacylg-
glycerol pathway, in the intestine about 25% of dietary TAG is
hydrolyzed into free glycerol and fatty acids during digestion
and absorption (21), and about 20–30% of TAG is synthesised
in the enterocyte via the glycerol 3-phosphate pathway (23).
Since AQP3, an aquaglyceroporin that enables water and
glycerol uptake, has been identified in the small intestine in
humans (7), we hypothesized that intravenously administered
\[^{3}H\]glycerol could cross the basolateral membrane of the
enterocyte, mix with free glycerol obtained from completely
hydrolyzed dietary TAG, and be incorporated into resynthe-
sized TAG. Glycerol kinase has been identified in rat intestinal
mucosa (9), and 14% of orally administered \[^{14}C\]glycerol was
identified in thoracic duct lymph TAG in rats, also demonstrat-
ing the presence of this enzyme in the small intestine (31). In
this study, glycerol enrichment was measurable in SF > 60 of
apoB-48-containing lipoproteins, demonstrating that this was a
viable method for labeling the intestinal lipoprotein pathway.
VLDL1 and VLDL2 TAG FCR have never been measured previously in a continuous feeding study. The higher VLDL1
TAG FCR during continuous feeding in study 1 compared with
study 2 may be due to the younger age of this group and the
mixed sexes (4 females, 2 males). Fasting VLDL TAG FCR
has been shown to be higher in young women than men (26).
Although CM FCR tended to be slightly higher than VLDL1
FCR in both feeding studies, this was not significant. There
was also no difference between VLDL1 and VLDL2 FCR in
either fed study. Hepatic and intestinal lipoproteins in the fed
state share the common hydrolysis pathway of lipoprotein
lipase (LPL), and several studies have suggested that lipopro-
teins compete for hydrolysis by LPL. In an in vitro study Fisher
et al. (13) showed that larger lipoprotein particles are prefer-
entially hydrolyzed by LPL. Bjorkegren et al. (6) showed that
a 60-min intravenous infusion of a chylomicron-like triglycer-
ide emulsion in healthy young men caused a 75–90% block in
the conversion of large VLDL apoB to small VLDL apoB,
suggesting there was competition between chylomicrons and
VLDL. However, Duez et al. (11) measured TRL apoB-100
and apoB48 kinetics in healthy subjects using a continuous
feeding protocol, with an infusion of \(^2\)H leucine, and also
showed that there was no difference in hepatic and intestinal
TRL particle FCRs (11). Using the same methodology, Pavlic
et al. (28) found no difference in VLDL1 apoB-100 or VLDL2
apoB-100 FCR. The different findings may reflect the different
study designs. The continuous feeding method allows a slow
rate of fat delivery, whereas in the Bjorkegren et al. (6) study
a large fat load was administered over 60 min, which would
have yielded much larger triglyceride-rich chylomicrons.

This study has shown that an intravenous glycerol tracer
combined with immunoaffinity chromatography can be used to
measure hepatic and intestinal TAG kinetics. The separation
of hepatic and intestinal lipoproteins is a very time-consuming
method and is dependent on the availability of specific apoB-
100 antibodies. The antibodies and the isotopic method are
costly, and the latter requires access to gas chromatography-
mass spectrometry. Despite these caveats, this methodology
may be a useful tool for understanding the abnormalities in
postprandial TAG kinetics in the metabolic syndrome and type
2 diabetes and the effect of different diets and treatments.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

F.S., M.S., F.S.-M., S.L., and Y.M. performed the experiments; F.S.,
F.S.-M., S.L., Y.M., R.H., and A.M.U. analyzed the data; F.S., M.S., F.S.-M.,
S.L., Y.M., R.H., and A.M.U. interpreted the results of the experiments; F.S.,
F.S.-M., and A.M.U. drafted the manuscript; F.S., M.S., F.S.-M., S.L., Y.M., R.H.,
and A.M.U. approved the final version of the manuscript; M.S., F.S.-M., S.L., Y.M., R.H.,
and A.M.U. edited and revised the manuscript; A.M.U. contributed to the conception and
design of the research.
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