A new method for the study of chylomicron kinetics in vivo

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Park, Yongsoon, Wayne J. Grellner, William S. Harris, and John M. Miles. A new method for the study of chylomicron kinetics in vivo. Am J Physiol Endocrinol Metab 279: E1258–E1263, 2000.—Our understanding of the metabolism of chylomicrons, the lipoprotein that transports dietary fat from the intestine to peripheral tissues, is incomplete. The present studies were conducted to determine whether a labeled intravenous lipid emulsion could be used to estimate chylomicron triglyceride (TG) rate of appearance (Ra) and thereby quantify the rate of intestinal fat absorption. After an overnight fast, healthy volunteers (n = 6) sipped a [3H]-labeled drink over 6.5 h at a rate of 175 mg fat·kg⁻¹·h⁻¹. Beginning at hour 5, an HPLC-purified, 14C-labeled lipid emulsion was infused intravenously for 90 min. During the study, plasma total and chylomicron TG concentrations increased from 100 ± 21 to 237 ± 40 mg/dl and from undetectable to steady-state levels of 35 ± 13 mg/dl, respectively. After a minor correction for VLDL contamination, tracer-determined chylomicron TG Ra was 175 ± 30 mg·kg⁻¹·h⁻¹, equal to the presumed ingestion rate. In summary, a radio-labeled intravenous lipid emulsion is able to accurately estimate chylomicron TG Ra and therefore can be used to measure in vivo fat absorption rates.

Triglyceride; lipid emulsion; absorption; appearance rate; tracer

Chylomicrons are lipoproteins secreted by the intestine after fat ingestion and are thus the primary vehicle for triglyceride (TG) absorption. Humans spend most of their time in the absorptive state, and an increased lipemic response to meals is associated with the development of atherosclerosis (1, 16, 17, 37, 38). For these reasons, a better understanding of chylomicron metabolism is needed.

Over 35 years ago, Nestel et al. (29) administered surgically obtained thoracic duct lymph chylomicrons intravenously to normal volunteers to study TG-rich lipoprotein metabolism. Although the use of native chylomicrons is ideal from a physiological standpoint, this method has obvious limitations related to its invasiveness and is thus not a practical option. Grundy and Mok (11) used a duodenal fat perfusion test to estimate chylomicron kinetics. This method is more practical than obtaining and injecting chylomicrons, but the procedure is unpleasant for the subject, requires fluoroscopic tube replacement, and could have unknown effects on gastrointestinal physiology (6).

Others have given a bolus injection of a large amount of unlabeled intravenous lipid emulsion (34). This approach is of limited value because, by markedly altering the TG pool size, it perturbs the parameters (clearance, half-life, and residence time) that it is attempting to measure. It may also result in significant, albeit transient, reticuloendothelial uptake of lipid (5, 6, 15, 36). A bolus injection of a trace amount of radiolabeled lipid emulsion allows the administration of less mass with less perturbation of the system (28, 33); however, there is controversy as to whether intravenous lipid emulsions are metabolized similarly to native chylomicrons (6, 28, 34). In this study, we labeled a commercially available lipid emulsion and administered it by constant intravenous infusion to trace chylomicron kinetics.

Method

Subjects

The study was approved by the St. Luke’s Hospital Institutional Review Board, and informed written consent was obtained from all participants. Six healthy, nonobese (body mass index 20–30 kg/m²) volunteers (ages 21–70) were studied (Table 1). Volunteers underwent a preliminary oral fat absorption test to exclude individuals with a low lipemic response (i.e., <50 mg/dl increase in serum TG 3 h after consuming 525 mg fat/kg).

Protocol

Subjects were asked to consume a low-fat evening meal (<30% energy from fat) at 1800 the night before the study and to refrain from alcohol and strenuous exercise for 48 h before the test. They reported to the Metabolic Research Unit at 0700 on the morning of the study. An intravenous sampling cannula was placed in a forearm vein and an infusion cannula in a contralateral vein; both were kept patent with controlled (30 ml/h) infusions of 0.9% NaCl. Subjects consumed a priming dose of a test drink labeled with [3H]triolein (see below), and 2 h later, they began to take a small portion

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of the drink every 15 min for the next 4.5 h to produce an overall rate of fat ingestion of 175 mg·kg⁻¹·h⁻¹. Between 5 and 6.5 h, an intravenous infusion of a [¹⁴C]triolein-labeled lipid emulsion, given by syringe pump (see Preparation of intravenous lipid emulsion), was initiated at ~0.3 μCi/min and was continued to the end of the study. The total amount of lipid infused was ~300 mg. Blood samples for total plasma TG analysis were drawn from the contralateral arm at 0 and 2 h and then every 30 min through 5.5 h. For determination of chylomicron TG concentration, [¹⁴C] and [³H] specific activities, fatty acid composition, and apolipoproteins, blood samples were taken every 8 min during the last 40 min of the study.

Laboratory Methods

Two labeled lipid emulsions were used in this study: 1) a [³H]triolein-labeled high-fat test drink, and 2) a [¹⁴C]triolein-labeled commercial lipid emulsion of relatively high specific activity for intravenous administration. Tritiated triolein was added to the test drink to permit an estimate of the extent of contamination of the chylomicron fraction by very low density lipoprotein (VLDL) TG. The labeled intravenous lipid emulsion was used to trace chylomicron TG metabolism in vivo.

Preparation of test drink. A vegetable oil-based drink containing soybean oil (54 g/l), safflower oil (54 g/l), dried nonfat milk (263 g/l), egg yolk phospholipid (0.18 g/l), and water was prepared by sonication (XL2020, Misonix, Farmingdale, NY). Finally, 300 μCi of [³H]triolein in 100 μl of ethanol (American Radiolabeled Chemicals, St. Louis, MO) were added and stirred into the drink. The drink provided 1.7 ± 0.02 mmol of ethyl ether-formic acid, 70:30:1, which was dissolved in ethyl ether. The final concentration was 0.34 ± 0.02 mmol/kg body weight.

Preparation of intravenous lipid emulsion. The [carboxyl-¹⁴C]triolein (American Radiolabeled Chemicals) was purified by thin-layer chromatography (TLC; silica gel G, hexane-ethyl ether-formic acid, 70:30:1) and then dissolved in ethanol. The [¹⁴C]triolein (60 μCi/400 μl in ethanol) was then added to 2 ml of 10% Liposyn (5% soybean oil, 5% safflower oil, 1.2% egg yolk phospholipid, and 2.5% glycerol) in a sterile vial, and the solution was gently agitated at 60°C for 5 min. The resulting emulsion was subjected to size-exclusion HPLC (25) indicated that a substantial fraction (~40%) of the radioactivity eluted at a point beyond endogenous chylomicrons in the chromatogram (Fig. 1, solid line), consistent with incorporation of radioactivity into TG-poor, phospholipid-rich vesicles known to be present in commercial lipid emulsions. These vesicles are present because of the excess phospholipid added to the emulsion to maintain stability (8). To isolate chylomicron-sized particles, the labeled emulsion was purified by HPLC by use of our previously published method for separating TG-rich lipoproteins (25) with a minor modification: 0.09% benzyl alcohol was added to the mobile phase to serve as a bacteriostatic agent. A 4-ml fraction, eluting between 32 and 40 min, was collected into a sterile vial containing 0.5 ml of unlabeled 10% Liposyn; the addition of unlabeled Liposyn restored the phospholipid excess to maintain the stability of the purified emulsion. This procedure was done twice, so that the final volume of labeled emulsion was 9 ml. The material was then autoclaved for 15 min. An HPLC radiochromatogram of the purified, sterilized emulsion demonstrated that the radioactivity coeluted with chylomicron-sized particles (Fig. 1, dashed line), indicating that there was no redistribution of radioactivity into phospholipid-rich vesicles. Particle-size distribution of the purified lipid emulsion was measured by dynamic light scattering (courtesy of Dr. Robert Lyons, Pharmacia and Upjohn, Clayton, NC) and was found to be similar (~0.34 μm) to that of human chylomicrons isolated by the triple spin technique described below in Chylomicron isolation (Fig. 2).

Radioactivity of chylomicrons and lipid emulsion. Aliquots of the chylomicron fraction (500 μl) and lipid emulsion (100 μl) were subjected to a three-step purification by use of a 3-ml ultracentrifuge tube and spin in an SW41 rotor at 16°C for 30 min at 25,000 rpm in a Beckman L7–65 ultracentrifuge (26). The top 1 ml was collected by aspiration and underlayered below 9 ml of distilled water and spun again under the same conditions. This procedure was repeated a third time, and the final purified chylomicron fraction was collected from the top of the tube. This triple spin approach was taken to remove as much VLDL as possible from the chylomicron fraction.

Chylomicron isolation. To isolate chylomicrons, 2 ml of plasma were underlayered beneath 8 ml of distilled water in an ultracentrifuge tube and spun in an SW41 rotor at 16°C for 30 min at 25,000 rpm in a Beckman L7–65 ultracentrifuge. The top 1 ml was collected by aspiration and underlayered below 9 ml of distilled water and spun again under the same conditions. This procedure was repeated a third time, and the final purified chylomicron fraction was collected from the top of the tube. This triple spin approach was taken to remove as much VLDL as possible from the chylomicron fraction.

Table 1. Subject characteristics

<table>
<thead>
<tr>
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<th>Women (n = 3)</th>
<th>Men (n = 3)</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>36 ± 3</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.7 ± 0.02</td>
<td>1.8 ± 0.02</td>
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<tr>
<td>Body weight, kg*</td>
<td>65 ± 7</td>
<td>89 ± 5</td>
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Values are means ± SE; *P < 0.01.


μl) were transferred to vials containing 10 ml Opti-fluor (Packard, Meriden, CT) and were counted in a Wallac 1410 liquid scintillation counter (Pharmacia, Gaithersburg, MD) with the use of appropriate quench curves established for dual label counting.

Quantification of apolipoprotein B-48 and B-100. To estimate the extent of VLDL contamination of the chylomicron fraction, the relative proportions of apolipoprotein (apo)B-100 (VLDL) and apoB-48 (chylomicron) were determined. Chylomicron fractions (1 ml) prepared from 12 ml of plasma were delipidated overnight with 10 ml of ethanol-ether (3:1) (35). The samples were centrifuged at 1,500 rpm for 20 min, and then the pellet was washed twice with ether and centrifuged again. After the ether was removed by aspiration, the pellet was solubilized in a buffer (pH 6.6) containing 0.125 M Tris, 10% glycerol, 30 mg/ml SDS, 15 mg/ml dithiothreitol, 10 mg/ml mercaptoacetate, and 0.025 mg/ml bromphenol blue and was heated for 3 min at 100°C. The protein concentration (24) of the sample was adjusted to 1 mg/ml sample buffer. Samples (50 μl) were loaded on 3–10% linear polyacrylamide slab gels; one gel was run for each plasma sample. Electrophoresis was carried out at a constant voltage of 50 V/gel for the first 30 min and then at 75 V/gel for 90 min (SX 250/260, Hoefer, San Francisco, CA) (19). The stacking gel was discarded, and the gradient gel was placed in staining buffer containing 0.25% Coomassie R-250 in methanol-acetic acid-water (5:1:5) for 18–22 h. After the staining buffer was discarded, the gel was placed in destaining buffer containing methanol-acetic acid-water (5:1:5) for ~5 h, with the buffer being changed every hour. Then, apoB-48 and apoB-100 were quantified by densitometric scanning (Sharp JX-330, Pharmacia Biotech) against the standard curve of apoB-100. The chromogenicities of human apoB-48 and apoB-100 have been shown to be equal (18). Standard apoB-100 was prepared from narrow-cut LDL (1.03–1.04 g/ml).

Calculations

Chylomicron TG rate of appearance (Rₐ) was calculated from the steady-state formula

\[
R_a = \frac{F}{SA}
\]

where \( R_a \) is the rate of appearance in milligrams per kilogram per hour, \( F \) is the tracer infusion rate, and \( SA \) is plasma chylomicron TG specific activity.

Statistical Analysis

We used a paired \( t \)-test to compare \(^{3}H \) specific activities of the drink and the chylomicron fraction and to compare tracer-determined \( R_a \) with the presumed fat absorption rate (175 mg·kg⁻¹·h⁻¹). A two-tailed \( P \) value of <0.05 was required for statistical significance.

RESULTS

Fasting plasma TG concentrations at baseline were 100 ± 21 mg/dl and increased to 237 ± 40 mg/dl by the end of the 6.5-h study (Fig. 3). Steady-state chylomi-
cron TG concentrations of 35 ± 13 mg/dl were achieved during the last 40 min of the study (Fig. 3). Chylomicron 3H and 14C specific activities were also at steady state, averaging 7,135 ± 520 dpm/mg for 3H and 3,079 ± 581 dpm/mg for 14C (Fig. 4). The 3H specific activity of the chylomicron fraction was 4% less than that of the drink (7,135 ± 540 vs. 7,411 ± 692, respectively). The amounts of palmitic (12 vs. 11%), stearic (4 vs. 4%), oleic (53 vs. 54%), linoleic (29 vs. 29%), and linolenic (2 vs. 2%) acids in chylomicrons and the drink were similar (data not shown). A typical PAGE gel is shown in Fig. 5. By use of the relative intensity of the B-100 and B-48 bands and with the relative TG content in VLDL and chylomicron particles taken into account, contamination of the chylomicron fraction by VLDL-TG was estimated to be 7 ± 1%. The tracer-determined chylomicron TG Rₐ was 187 ± 30 mg·kg⁻¹·h⁻¹ and was similar to the predicted absorption rate of 175 mg·kg⁻¹·h⁻¹ (Fig. 6). When 3H specific activity was used to correct 14C specific activity for the presence of unlabeled VLDL-TG in the chylomicron samples, chylomicron Rₐ was 175 ± 30 mg·kg⁻¹·h⁻¹ (Fig. 6).

**DISCUSSION**

This study presents a practical method for preparing a radiolabeled TG lipid emulsion for intravenous use in humans. It also demonstrates that the labeled emulsion produces excellent estimates of chylomicron Rₐ during steady-state absorption of a fat drink.

Previous methods for determining chylomicron kinetics in vivo were fraught with technical difficulties or used approaches of dubious physiological relevance (11, 29, 34). The goal of the present investigation was to develop a practical and reliable method for the study of chylomicron metabolism. We hypothesized that the tracer-determined Rₐ of chylomicron TG is equal to the presumed absorption rate during steady-state absorption of a test meal. Several tools were needed to test this hypothesis: 1) a chylomicron-like labeled lipid emulsion, 2) a method to produce steady-state chylomicron TG concentrations, and 3) a technique to isolate chylomicrons and determine the extent of VLDL contamination.

Commercially available lipid emulsions have been shown to contain not only TG-rich particles but also phospholipid-rich, TG-poor vesicles (21, 22). When radiolabeled triolein was added to the emulsion, we found that ~40% of radioactivity was incorporated into these liposomes. Because these particles are smaller than (and presumably metabolized differently from) the chylomicrons we wished to trace, it was necessary to remove them (12, 21, 22). This was accomplished with HPLC, which isolated chylomicron-sized TG-rich particles of relatively high specific activity. It was important that the particles in the infused emulsion be of similar size to chylomicrons, because the affinity of lipoprotein lipase (LPL) for TG-rich lipoproteins is influenced by particle size (14, 31).

The chylomicron isolation technique is critically important, because contamination of this fraction by VLDL-TG would result in an underestimate of chylomicron TG specific activity and, hence, an overestimate of Rₐ. The separation of chylomicrons from VLDL is based on an operational definition rather than on a true physiological distinction. It is impossible to completely recover 100% pure chylomicrons by either centrifugation, due to the similarity of densities between chylomicrons and VLDL (~0.93 vs. ~0.97 g/ml) (20,

Fig. 4. The 3H and 14C TG specific activities during steady-state triglyceridemia. The former represent chylomicrons derived from the labeled test drink and the latter the labeled infused lipid emulsion.
32), or by immunoaffinity chromatography with the use of monoclonal antibodies 4G3 and 5E11 (4, 20). The routine detection of apoB-100 in the S^c_1 >400 fraction (4, 7, 20, 32) confirms that some VLDL contamination of chylomicron isolates is inevitable. Because a relatively pure chylomicron preparation is critical for the accurate quantitation of chylomicron TG metabolism, we tested the purity of the chylomicron fraction isolated by our triple-spin method using three approaches: comparison of chylomicron and drink specific activities, comparison of chylomicron and drink fatty acid composition, and the apoB-100-to-apoB-48 ratio in the chylomicrons. Because chylomicron TG specific activity in a pure preparation should be the same as the TG specific activity in the drink, any discrepancy between the two would indicate VLDL-TG contamination. We found that the triple-spin method isolates chylomicrons with a specific activity only 4% less than that of the drink TG. The second approach was a comparison of chylomicron TG fatty acid composition with that in the drink; they were almost identical. The similarity of both specific activity and fatty acid composition suggests that fatty acid exchange in the enterocyte is minimal, which is not surprising, because previous studies have shown that enterocyte fatty acids derived from plasma are poorly utilized for chylomicron TG formation (23). Finally, the TG contribution from chylomicrons and VLDL in the specimens was estimated to be 93% and 7%, respectively, based on the ratios of apoB-100 and apoB-48 on PAGE gels (3). The relative concordance in estimates of VLDL-TG contamination with these three approaches supports a conclusion that contamination using our technique was relatively minor. We used the 96% purity figure to correct chylomicron TG Ra, because it was derived from a direct measurement (specific activity) and did not require the assumptions necessary when estimating VLDL TG content from apoB-100-to-apoB-48 ratios.

The “sipping” procedure used in these studies was successful in producing a plateau in whole plasma and chylomicron TG concentrations for the last 40 min of the study. This approach of periodic consumption of a high-fat drink is easy to carry out and eliminates the concern about altered digestive processes when an intraduodenal feeding tube is used. Nguyen et al. (30) also used periodic feedings and found a pattern of plasma TG comparable to gastric infusion. The TG response to fat ingestion in the study of Nguyen et al. was probably because of lower fat ingestion rates (65 vs. 175 mg·kg^-1·h^-1). Because intestinal absorption of dietary fat is essentially complete (2), the rate of absorption can be assumed to equal the ingestion rate at steady state. This procedure therefore achieves a known input of chylomicron TG, a condition required to test the tracer technique. It is not clear from previous studies whether or not the metabolism of the chylomicron-like particles in intravenous lipid emulsions is similar to that of native chylomicrons. Results from intravenous fat tolerance tests suggest that the clearance of TG after an intravenous bolus of unlabeled lipid emulsion is 100% (34) to 200% (6) slower than the clearance of a bolus tracer dose of radiolabeled lipid (28). However, the use of large doses of unlabeled lipid for the study of TG metabolism is likely to produce results that are confounded by the method itself. In other words, the intravenous fat tolerance test raises plasma TG concentrations and thus affects the parameters (half-life, clearance, residence time) that it attempts to measure. This would apply to any substance whose removal is saturable and therefore does not follow first-order kinetics. For this reason, tracer doses of labeled lipid are preferable to large doses of unlabeled lipid. Hultin et al. (14) gave rats bolus injections of radiolabeled harvested chylomicrons or radiolabeled lipid emulsion and found that the lipid emulsion was cleared 35–40% more slowly than the native chylomicrons. However, the emulsion used in those studies was prepared locally and had a significantly different composition and a larger particle size (0.4 μm) than native chylomicrons or commercial emulsions (14). Nakandakare et al. (28) gave a bolus injection of a lipid emulsion labeled with [3H]triolein to normolipidemic volunteers and estimated the chylomicron residence time to be 5.9 min, similar to the residence time (6.5 min) estimated from a duodenal fat perfusion study (11). The small differences in these results may have been due to the differences in the prevailing TG concentrations in the two studies (118 vs. 182 mg/dl); because LPL is saturable, half-life and residence time would be expected to be longer at higher TG concentrations (10). Our data demonstrate that a radiolabeled lipid emulsion is metabolized similarly to native chylomicrons. The uncorrected, tracer-determined chylomicron TG Ra was 104% of the presumed fat absorption rate (175 mg·kg^-1·h^-1), and even more accurate estimates were obtained after correction for VLDL-TG contamination. Although tracer-determined and presumed chylomicron TG Ra values were essentially identical, it should be acknowledged that there was some variability around the mean. This is likely due both to the finite precision of the analytical methods used and to variability between individuals in actual rates of fat absorption during the sampling interval.

In summary, a radiolabeled intravenous lipid emulsion is metabolized by LPL similarly to native chylomicrons and provides excellent estimation of chylomicron TG Ra in humans. We conclude that this tracer technique should be a useful tool for the study of chylomicron TG metabolism. This technique could be applied to investigate the effects of diets, drugs, disease, and physiological perturbations on chylomicron TG kinetics in the future.

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