Effects of triacylglycerol and diacylglycerol oils on blood clearance, tissue uptake, and hepatic apolipoprotein B secretion in mice

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Abstract **Prior studies have suggested that FAs liberated in the small intestine from ingested 1,3-diacylglycerol (DAG) are inefficiently incorporated into triglyceride (TG) in enterocytes, with less chylomicron TG entering the circulation postprandially. We found less TG, but more monacylglyerol and DAG, with similar total acylglycerol in newly secreted chylomicrons after oral DAG or triacylglycerol (TAG). However, clearance of DAG-chylomicrons was more rapid than that of TAG-chylomicrons; this was associated with more efficient in vitro LPL-mediated lipolysis of DAG-derived chylomicrons. Intravenously infused DAG was also cleared faster than TAG in normal mice, via both LPL-mediated lipolysis and apolipoprotein E (apoE)-dependent hepatic uptake. Infusions of TAG, but not DAG, increased plasma TG levels. Greater delivery of DAG-derived FA to the liver during infusion of DAG led to greater TG secretion versus TAG; this allowed the maintenance of similar hepatic TG levels after DAG and TAG infusions. Of note, apoB secretion was similar after DAG versus TAG, indicating the assembly of larger very low density lipoproteins after DAG.** **In conclusion, reduced plasma TG levels, after oral or intravenous DAG, result from more efficient clearance of DAG by both LPL lipolysis and apoE-mediated hepatic endocytosis. DAG emulsions may by useful for intravenous nutrition in people with preexisting hypertriglyceridemia.**—Yasunaga, K., S. Saito, Y-L. Zhang, A. Hernandez-Ono, and H. N. Ginsberg. **Effects of triacylglycerol and diacylglycerol oils on blood clearance, tissue uptake, and hepatic apolipoprotein B secretion in mice.** ***J. Lipid Res.*** **2007.** 48: **1108–1121.**

Supplementary key words triglycerides • lipoprotein metabolism • chylomicron metabolism

Recent studies in animals and humans suggest that oral ingestion of diacylglycerol (DAG), specifically 1,3-DAG, results in lower postprandial triglyceride (TG) levels in plasma compared with levels after ingestion of triacylglycerol (TAG) with similar FA composition (1–5). These differences occur despite the comparable digestibility and energy content of the two emulsions (6). A potential explanation for this difference was generated by studies in which the lymphatic transport of chylomicron after 1,3-DAG ingestion was significantly delayed and reduced, presumably as a result of poor reesterification of FA onto either 1-monoacylglycerol (MAG) or glycerol in the intestinal mucosa (7–9). However, the metabolism of the plasma chylomicrons and their remnants generated by oral administration of DAG and TAG emulsions has not been investigated. Furthermore, there is no information regarding the metabolism of DAG and TAG emulsions after intravenous administration.

Lipid emulsions, primarily composed of TAG and phospholipids, have been used widely in parenteral nutrition for several decades. The metabolic pathways for processing lipid emulsions injected intravenously are similar to those for chylomicrons and VLDL: apolipoproteins are acquired from circulating lipoproteins and are taken up by tissues, mainly the liver, after hydrolysis by LPL (10, 11). The most commonly used emulsion in clinical settings, Intralipid, has a FA composition derived mainly from soybean oil. Studies in animals and humans have been conducted to investigate the effects of emulsions with differing FAs used as core lipid components. For example, lipid emulsions rich in medium-chain TAG were demonstrated to be more efficiently hydrolyzed by LPL or HL than emulsions with long-chain TAG, resulting in rapid clearance of medium-chain TAG emulsions from blood (12, 13). On the other hand, lipid emulsions rich in ω-3 FAs from fish oil are less efficiently hydrolyzed by LPL but cleared more efficiently as whole particles compared with ω-6 emulsion (14–16).

By contrast, comparisons of the metabolism of intravenous lipid emulsions with similar FAs, but with differing

Manuscript received 11 December 2006 and in revised form 29 January 2007.
Published, JLR Papers in Press, February 3, 2007.
DOI 10.1194/jlr.M600524-JLR200

Abbreviations: AG, acylglycerol; AOX, acyl-coenzyme A oxidase; apoB, apolipoprotein B; CE, cholesteryl ester; CEt, cholesterol oleoyl ether; CPT-1, carnitine palmitoyltransferase-1; DAG, diacylglycerol; DO, diolein; MAG, 1-monoacylglycerol; PhP, postheparinized plasma; PPARα, peroxisome proliferator-activated receptor α; TAG, triacylglycerol; TG, triglyceride; TO, triolein.

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acylglycerol (AG) structures (i.e., TAG and DAG), are lacking. Recent studies in rodents with DAG have indicated that it may be metabolized by distinct pathways in the gastrointestinal tract when administered orally (2, 7, 9, 17, 18). In addition, several physicochemical properties of DAG with respect to the water-oil interface and emulsification have been reported to differ from those of TAG as a result of DAG's greater hydrophilicity (19).

In this study, our first goal was to determine the basis for the findings of prior studies of lower postprandial TG levels after oral administration of DAG emulsions. Was there reduced intestinal transport of chylomicron AG in animals gavaged with DAG versus TAG? Or was the plasma metabolism of chylomicrons produced after ingestion of 1,3-DAG more efficient compared with the metabolism of chylomicrons generated by ingestion of a TAG emulsion? Our second goal was to investigate, in detail, the metabolism of intravenously administered DAG emulsions, particularly with regard to their intravascular metabolism and delivery of FAs to tissues. Finally, because we recently observed differential regulation of hepatic apolipoprotein B (apoB) lipoprotein assembly and secretion by intravenous infusion of albumin-bound FAs compared with a TAG emulsion (20), it was of interest to investigate the effects of intravenous infusions of TAG and DAG emulsions on hepatic TG accumulation and the secretion of VLDL.

MATERIALS AND METHODS

Chemicals

[^{3}H]cholesterol oleoyl ether ([^{3}H]CEt) and [1-^{14}C]triolein ([^{14}C]TO) were purchased from Amersham Pharmacia Biotech. [1-^{14}C]diolein ([^{14}C]DO) was purchased from American Radioactive Chemicals, Inc. [^{35}S]methionine and [^{32}P]ribonucleic acid were purchased from Perkin-Elmer Life Science (Wellesley, MA). BSA in fatty acid-free grade was purchased from ICI. Egg yolk phosphatidylcholine, glycerin, Triton WR1339, and Triton X-100 were purchased from Sigma-Aldrich.

Animals

Male C57BL/6J mice, age 12–20 weeks, were purchased from the Jackson Laboratory. ApoE-deficient mice were kindly provided by Dr. Neil S. Shachter (Columbia University). All mice were maintained in Columbia's animal facility in a 12 h light/12 h dark cycle and fed a regular rodent chow diet.

Plasma lipid determination

Plasma TG levels were measured with a commercial kit from Wako Chemicals. In studies in which DAG emulsions were injected, the plasma total AG level was calculated by converting the TG level obtained from the commercial kit to DAG based on the molar ratio. Plasma FA concentrations were measured using a commercial kit from Wako Chemicals.

Test oils

DAG oil was prepared by esterification of FA derived from natural plant edible oil with MAG or glycerol in the presence of immobilized lipase (21, 22) and purified further with open column liquid chromatography (23). TAG oil was prepared by mixing rapeseed, safflower, and perilla oils to achieve a FA composition comparable to that of the DAG. The ester distributions of AGs and the FA compositions of TAG and DAG (by weight) were determined by gas chromatography. The weight fractions of FA in TAG and DAG oil were calculated as 95.6% and 91.3%, respectively (6).

Preparation of radiolabeled emulsion

Twenty percent TAG and DAG emulsions by weight were prepared using ultrasonication as reported previously (12). In brief, the oil phase, composed of 12 mg of egg-phosphatidylcholine and 2 g of either TAG or DAG, was dispersed in the water phase, composed of 1 g of BSA, 250 mg of glycerol, and 6.6 g of doubly distilled water by homogenization (homogenizer model 398; Biospec Products, Inc.). Subsequently, the dispersion was homogenized in a cooling cell with an ultrasound sonicator (type 853973/1, Braun-Sonic U) for 10 min at the power setting of 200 W. Mean particle sizes of the emulsions were determined by laser light-scattering spectrometry (SALD-2100; Shimadzu, Kyoto, Japan). To trace both emulsion particle and emulsion FA catabolism, each emulsion was labeled with [^{3}H]CEt and either [^{14}C]TO or [^{14}C]DO, as described previously (12). Briefly, [^{3}H]CEt and either [^{14}C]TO or [^{14}C]DO were added to a small glass vial and dried under N_2 gas. One milliliter of each emulsion was added to the vial and mixed thoroughly, and the emulsion was sonicated three times on ice for 20 s each at the power setting of 40 W using the Branson Sonifier Cell Disrupter (model W185; Branson Scientific, Melville, NY) to incorporate the labeled lipids into the core of emulsion particles. Emulsions were stored at 4°C until use.

Distribution of [^{14}C]FAs into plasma lipids after oral administration of radiolabeled TAG or DAG emulsions

Two hours after gavage with radiolabeled TAG or DAG emulsion in Triton WR1339-treated mice, blood was collected from the retro-orbital plexus for isolation of plasma. Plasma lipids were extracted by modification of the Folch extraction (24), and the extracted lipids were identified by TLC.

Determination of chylomicron secretion after oral administration of TAG or DAG oil

Overnight (14 h)-fasted wild-type mice were gavaged with 400 μl of emulsion containing 80 mg of either TAG or DAG labeled with 1–2 μCi of [^{14}C]TO or [^{14}C]DO, respectively. In some studies, overnight (14 h)-fasted wild-type mice were anesthetized with 3.3 μl/g body weight of ketamine (15 mg/ml) and xylazine (3 mg/ml) and then injected with 500 mg/kg Triton WR1339 in 0.9% sodium chloride via femoral vein 15 min before being gavaged with 400 μl of emulsion containing 80 mg of either radiolabeled TAG or DAG. Retro-orbital blood was drawn before and at several time points after gavage for measurement of plasma AG levels and ^{14}C radioactivity.

Isolation of chylomicrons

In other studies in which radiolabeled emulsions were administered by gavage after injection of Triton WR1339, as described above, plasma samples were obtained 2 h later and chylomicrons were isolated by ultracentrifugation at 40,000 rpm for 30 min at 4°C in a TL-100 Ultracentrifuge (Beckman Coulter, Palo Alto, CA) using a TLA100.3 rotor.

Chylomicron clearance study in vivo

Overnight (14 h)-fasted mice were anesthetized with 3.3 μl/g body weight of ketamine (15 mg/ml) and xylazine (3 mg/ml) and then received a bolus injection of chylomicrons, generated and isolated as described above, via the femoral vein. Blood samples were obtained at several time points, and preinjection and postinjection plasma AG levels and ^{14}C radioactivity were measured.

Hydrolysis rates of TAG and DAG emulsions using LPL in vitro assay

Rates of hydrolysis of TAG and DAG emulsions were assessed in vitro using postheparinized human plasma as described previously with some modifications (25). Briefly, postheparinized human plasma was obtained 15 min after an intravenous injection of 60 U/kg heparin. After separation of plasma at 4°C, samples were stored at −80°C until assay. TAG and DAG emulsions labeled with [^{14}C]TO and [^{14}C]DO, respectively, were incubated with postheparinized human plasma for 1 h at 25°C. The rate of hydrolysis was determined by the quantity of [^{14}C]FA released from the emulsion.

To assess the rate of hydrolysis of chylomicrons isolated after oral administration of TAG or DAG emulsions, chylomicrons were incubated with postheparinized mouse plasma for 1 h at 25°C and the amount of [1-^{14}C]FA released was determined.

Blood clearance and tissue uptake of emulsions after intravenous bolus injections

Mice were anesthetized with inhalant anesthetics (Metofane; Mallinckrodt Veterinary, Inc., St. Louis, MO) and received 100 μl of emulsion containing 20 mg of either TAG or DAG labeled with 0.8 μCi of [^{3}H]CEt or 0.15 μCi of either [^{14}C]TO or [^{14}C]DO by bolus injection via femoral vein. Blood samples were collected into heparinized capillary tubes from the retro-orbital plexus. Clearance of emulsion particles and FAs derived from the TAG and DAG emulsions was assessed by measuring the level of [^{3}H]CEt and either [^{14}C]TO or [^{14}C]DO over time (30 min for CEt and 5 min for FAs). Plasma lipid levels were assessed at the same time points. Mice were euthanized and cleared of whole blood by flushing with 20 ml of phosphate-buffered saline through the left ventricle immediately after the final blood sampling. The organs (heart, lung, liver, spleen, and kidney) and tissues (gastrocnemius and femoris muscles and epididymal fat) were collected, weighed, and homogenized in 5 ml of phosphate-buffered saline using a Polytron Tissue Disrupter (Kinematics AG, Zurich, Switzerland). One milliliter aliquots of the homogenates were added to 3.5 ml of scintillation fluids, and radioactivity was counted to determine tissue uptake. Tissue uptake was expressed as the percentage of total recovered radioactivity, including that from blood at the final time point. The whole blood volume for each mouse was calculated as 4.9% of its body weight. Fat and muscle mass were calculated as 15% and 42% of body weight, respectively.

Constant intravenous infusions of emulsion

Mice were anesthetized with 3.3 μl/g body weight ketamine (15 mg/ml) and xylazine (3 mg/ml), and a catheter was inserted into the jugular vein using a dissecting microscope as described previously (20). The mice were allowed to recover for 2–3 days before experiments were performed. The tubing was flushed several times with saline during that period. On the morning of the experiment, food was removed and the catheter was connected through polyethylene tubing to a Harvard Compact Infusion Pump (Harvard Apparatus, Cambridge, MA). TAG or DAG emulsions were infused at the rate of 2.5 μl/min for 6 h. Blood samples were obtained from the retro-orbital plexus before and at various time points during the infusion for measurement of plasma TG and FA.

FA delivery to the liver by infusion of TAG or DAG

Delivery of FAs to the liver during constant infusions of 5% TAG or DAG was determined by rapid injection of 0.125% TAG or DAG, radiolabeled with 0.15 μCi of [^{14}C]TO and [^{14}C]DO, after 4.5 h infusions. The choice of 0.125% radiolabeled emulsions was based on our goal of introducing quantities of each source of FA into the circulation by bolus injection that would approximate the quantities delivered during 1 min of the infusion of 5% emulsions. Five minutes after bolus injection of the radiolabeled emulsions, animals were euthanized and organs and tissues were collected as described above.

Determination of hepatic lipids after TAG or DAG infusions

At the end of 6 h infusions, or 5 min after bolus injections of ^{14}C-radiolabeled emulsions, livers were collected for the measurement of hepatic lipids. Briefly, snap-frozen liver tissue (∼500 mg) was homogenized and extracted twice with chloroform-methanol (2:1, v/v) solution (24). The organic layer was dried under nitrogen gas and resolubilized in chloroform. An aliquot was suspended in an aqueous solution containing 2% Triton X-100 for the determination of TG mass (26). Total liver protein was extracted using Tissue Protein Extraction Reagent (T-PER™, code 78510; Pierce). Protease Inhibitor Mixture (code 1873580; Roche Diagnostics) was added into the liver protein extraction to prevent protein degradation. Liver TG levels were expressed as micrograms of TG per milligram of liver protein. The remaining extraction was applied to the silica gel plate and developed with hexane-ethyl ether-acetic acid (50:50:1) in a sealed glass chamber, developed, and exposed to saturated iodine vapor to stain lipid spots. The spots were scraped and suspended in scintillation fluid for the determination of radioactivity. Each lipid was expressed as the percentage of total recovered radioactivity.

Determination of TG and apoB secretion rates

After 6 h infusions, in vivo secretion rates of TG and apoB were determined as described previously (27). Mice were injected intravenously with a mixture of 200 μCi of [^{35}S]methionine and 500 mg/kg Triton WR1339 in 0.9% sodium chloride. Both lipolysis and tissue uptake of lipoproteins are completely inhibited in mice under these conditions (28), and the accumulation of TG and [^{35}S]apoB in plasma after injection of Triton WR1339 can be used to estimate rates of secretion of each component of VLDL (29). Blood samples were collected at the end of the 6 h infusion (0 min, preinjection) and at 30, 60, 90, and 120 min after injection of Triton WR1339 for the determination of TG levels. Five microliters of whole plasma sample from the 120 min time point was subjected to 4% SDS-PAGE, and autoradiography with densitometry was used to estimate the accumulation of [^{35}S]apoB in plasma over 2 h, a measure of the secretion of newly synthesized apoB into plasma over that period of time.

Hepatic gene expression

Total cellular RNA was isolated from livers of mice infused with saline, TAG, or DAG emulsions using TRIzol Reagent (Life Technology) according to the protocol provided by the company. RNase protection assays were performed as described previously (30). The RNA probes were generated by amplification of target genes from total RNA of male C57BL/6J mice by RT-PCR (30). Each PCR product was cloned into PCRII vector using a TA cloning kit obtained from Invitrogen. DNA sequences of these clones were verified by DNA sequencing using an ABI 377 automatic DNA sequencer (Perkin-Elmer Life Science).

Statistical analysis

Means and SD values are presented. Statistically significant differences ($P < 0.05$; two-tailed) of the mean values between two groups were assessed by Student's *t*-test and repeated two-way ANOVA.

RESULTS

Lipid compositions and particle size distributions of emulsions

Because of potential effects of size and composition on their plasma metabolism (31–33), we carefully characterized the emulsions used in our studies. The results (**Table 1**) demonstrate that the purity of the DAG and TAG oils, with regard to AG composition, were 99.3% and 98.6%, respectively. The proportions of the 1,2- and 1,3-isoforms in the DAG oil were 38% and 61%, respectively. Additionally, the FA composition of the two oils was almost identical (Table 1). Particle size distributions of the two emulsions were also essentially identical, with mean diameters of 554 ± 156 nm in TAG and 534 ± 133 nm in DAG (**Fig. 1**). These particle sizes are comparable to the commercially available Intralipid and correspond to the size of chylomicrons.

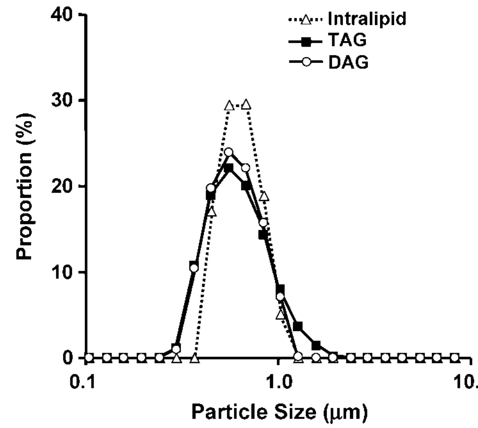

Fig. 1. Particle size distribution of triacylglycerol (TAG) and diacylglycerol (DAG) emulsions. TAG and DAG emulsions were prepared with an ultrasonic homogenizer. Particle size distributions of each type of emulsion were measured with laser light-scattering spectrometry and expressed as percentage content as a function of the diameter of the particle (logarithmic scale).

Distribution of radiolabeled FAs in plasma after oral administration of TAG or DAG emulsions together with Triton WR1339

To characterize newly assembled chylomicrons after oral administration of either DAG or TAG, we injected Triton WR1339 intravenously 15 min before gavage of each emulsion. Triton WR1339 blocks the catabolism of all lipoproteins in the plasma (29) and allowed us to examine the impact of nascent chylomicrons produced by DAG and TAG emulsions. TLC revealed that although there was a small but significantly lower plasma TAG level after DAG administration compared with TAG administration (please note that the references for the height of the bars for TAG and AG in **Fig. 2** are on the right side of the figure), there were 3-fold higher levels of 1,3-DAG and 1.5-fold higher levels of MAG and FA (Fig. 2) (TAG vs. DAG: 1,3-DAG, 0.63 ± 0.19 vs. 1.86 ± 0.47%, $P < 0.001$; MAG, 0.26 ± 0.09 vs. 0.39 ± 0.15%, $P < 0.05$; FA, 1.73 ± 0.73 vs. 2.63 ± 0.66%, $P < 0.01$; TAG, 92.93 ± 1.59 vs. 90.41 ± 2.25%, $P < 0.01$). The increases in MAG and DAG levels compensated for the small reduction in the major core lipid, TAG; therefore, total chylomicron AG levels were the same after ingestion of the two emulsions. There was no difference in phospholipid, 1,2-DAG, cholesteryl ester (CE), or total AG (MAG + 1,2-DAG + 1,3-DAG + TAG) in plasma after TAG or DAG administration (Fig. 2) (TAG vs. DAG: phospholipid, 0.99 ± 0.35 vs. 1.33 ± 0.57%; 1,2-DAG, 1.51 ± 0.31 vs. 1.63 ± 0.50%; CE, 1.95 ± 0.57 vs. 1.75 ± 0.49%; total AG, 95.32 ± 1.25 vs. 94.29 ± 1.32%). These results indicated that there were modest but significant differences in the lipid composition of chylomicrons generated by oral ingestion of DAG or TAG emulsions.

TABLE 1. Acylglycerol and fatty acid compositions of the test oils

Variable	Diacylglycerol	Triacylglycerol
Acylglycerols (%)		
Monoglycerides	0.0	0.0
Diglycerides	99.3	1.4
1,2-Diglycerides	38.4	
1,3-Diglycerides	60.9	
Triglycerides	0.7	98.6
Fatty acids (%)		
C14:0	0.06	0.05
C16:0	4.21	4.24
C18:0	1.90	1.97
C18:1	56.61	61.14
C18:2	21.94	20.37
C18:3	10.94	10.75
C20:0	0.56	0.65
C20:1	0.31	0.37
C22:0	0.08	0.07
C22:1	0.13	0.14

Acylglycerol and fatty acid compositions of the test oils were measured with gas chromatography and expressed as contents by weight.

Metabolism of AGs and FAs in plasma after oral administration of TAG or DAG emulsions

We next examined the plasma metabolism of the chylomicrons generated by gavage of TAG or DAG radiolabeled with [^{14}C]TO or [^{14}C]DO, respectively. Plasma total AG and ^{14}C levels at 2 h time points were significantly lower after DAG emulsion compared with TAG emulsion (**Fig. 3A**) (TAG vs. DAG: plasma AG level, 117.6 ± 32.7 vs. 80.9 ± 27.7 mg/dl, $P < 0.05$; recovery of ^{14}C activity in plasma, 1.44 ± 0.43 vs. 0.93 ± 0.34%, $P < 0.05$). However, when each emulsion was given by gavage at 15 min after intravenous injection of Triton WR1339, there was no significant difference in the postprandial increases in AG levels between DAG and TAG (Fig. 3B). Together, these two experiments suggest that chylomicron assembly and secretion was normal after DAG but that once

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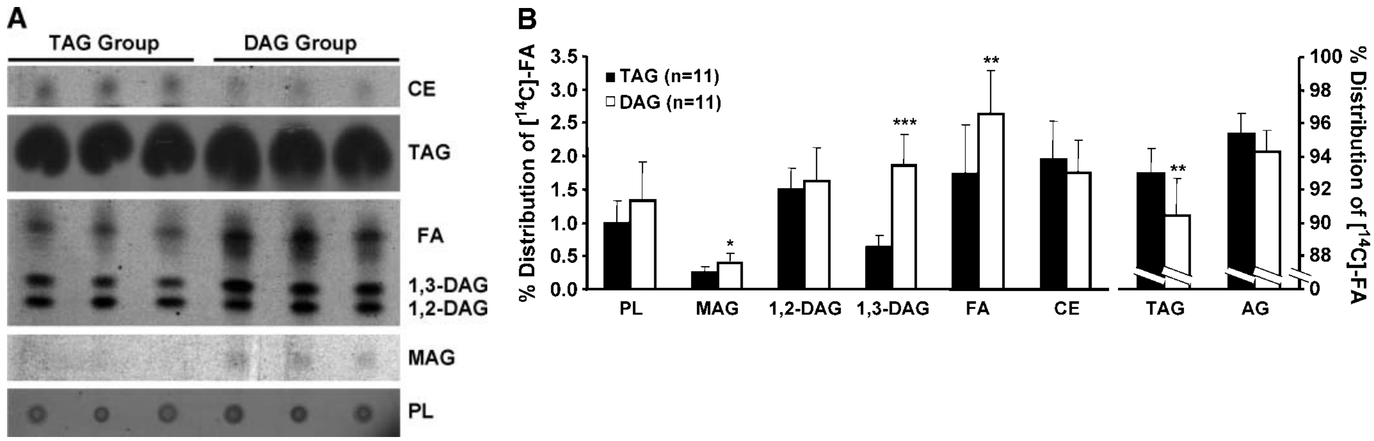

Fig. 2. Distribution of plasma lipids after gavage of TAG or DAG emulsions and intravenous injection of Triton WR1339. Overnight (14 h)-fasted mice were gavaged with 400 μl of emulsion containing 80 mg of either TAG or DAG labeled with 1–2 μCi of [^{14}C]triolein ([^{14}C]TO) or [^{14}C]diolein ([^{14}C]DO), respectively. Mice received 500 mg/kg Triton WR1339 in 0.9% sodium chloride via the femoral vein 15 min before being gavaged. Two hours after gavage, blood was collected from the retro-orbital plexus, plasma lipids were extracted by modification of the Folch procedure (24), and the extracted lipids were identified by TLC. A: A representative TLC plate. B: Distribution of ^{14}C-radiolabeled plasma lipids; in the presence of Triton WR1339, all radiolabeled lipids are assumed to be in chylomicrons produced from the emulsion administered. Note that the left and right y axes differ markedly: the left y axis is for phospholipid (PL), 1-monoacylglycerol (MAG), 1,2-DAG, 1,3-DAG, FAs, and cholesteryl ester (CE), whereas the right y axis is for TAG and total acylglycerol (AG), which comprises MAG, 1,2-DAG, 1,3-DAG, and TAG. All of the data are means ± SD and are expressed as percentage of total radioactivity in plasma (11 mice in each group). The asterisks denote statistically significant differences between TAG and DAG groups (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

DAG-chylomicrons had entered plasma, they were hydrolyzed more effectively by LPL compared with TAG-chylomicrons. Consistent with these results, and with the TLC results in Fig. 2, the increase in plasma FA levels after DAG administration with Triton WR1339 treatment was significantly greater than that after TAG administration (Fig. 3C, right panel) ($P < 0.05$ by ANOVA), whereas there were no significant differences in plasma FA levels after gavage of DAG and TAG without Triton WR1339 treatment (Fig. 3C, left panel). This suggests that FA flux into the circulation from the small intestine was increased after DAG administration.

In vitro and in vivo metabolism of chylomicrons isolated after oral administration of TAG or DAG emulsions together with Triton WR1339

To extend our findings suggesting better lipolysis of chylomicrons from DAG, we conducted in vitro studies of LPL-mediated lipolysis. Plasma chylomicrons generated after gavage of DAG or TAG emulsions were isolated from plasma of mice treated with Triton WR1339 and incubated with varying amounts of postheparinized plasma (PhP) obtained from the other mice. After a 1 h incubation in vitro, DAG-chylomicrons were hydrolyzed more effectively by PhP than TAG-chylomicrons in a dose-dependent manner (**Fig. 4A**). This result demonstrates that the rate of lipolysis of lipids in the DAG-generated chylomicrons was 1.3-fold higher than that in the TAG-generated chylomicrons (Fig. 4A) (TAG vs. DAG: 9.1 vs. 12.1 μmol FA/ml PhP/h, $P < 0.01$). Next, isolated chylomicrons generated by each emulsion were injected into other male mice via the femoral vein to observe their clearance from the circulation. DAG-chylomicrons were cleared significantly faster than TAG-chylomicrons in the recipient mice (Fig. 4B) ($P < 0.001$ by ANOVA). The fractional catabolic rate of chylomicrons in the DAG recipient group was 1.4-fold higher than that in the TAG recipient group (TAG vs. DAG: 6.50 ± 1.86 vs. 9.04 ± 2.87 pool/h, $P < 0.05$).

Blood clearance and tissue delivery of emulsions after bolus injection

The previous experiments indicated that, rather than differences in the assembly and secretion of chylomicrons after oral administration of TAG and DAG emulsions, it was the differences in the chylomicrons themselves that led to lower postprandial plasma AG levels after ingestion of DAG. In particular, the data suggested that chylomicrons entering plasma after DAG administration were better substrates for LPL. To examine this more directly, without any potential confounding related to the digestion or absorption of each emulsion, or to the assembly of chylomicrons after gavage with each emulsion, we determined the clearance and tissue delivery of intravenously injected TAG and DAG emulsions.

The clearance of both AGs and whole emulsion particles in normal mice was determined after bolus injection of either 20% TAG or DAG emulsions radiolabeled with 0.8 μCi of nonhydrolyzable core lipid [^{3}H]CEt in a 100 μl volume. The results (**Fig. 5A**) demonstrate that AGs in the DAG emulsion were cleared faster than AGs in the TAG emulsion. A similar pattern was observed for particle clearance, as assessed by the plasma levels of [^{3}H]CEt, indicating that the DAG particles were also cleared faster from blood than the TAG particles (Fig. 5B). The fractional catabolic rate of [^{3}H]CEt in blood after injection of DAG emulsion was more than twice that of the TAG emulsion (TAG vs. DAG: 2.53 ± 1.1 vs. 5.17 ± 1.2 pool/h, $P < 0.01$).

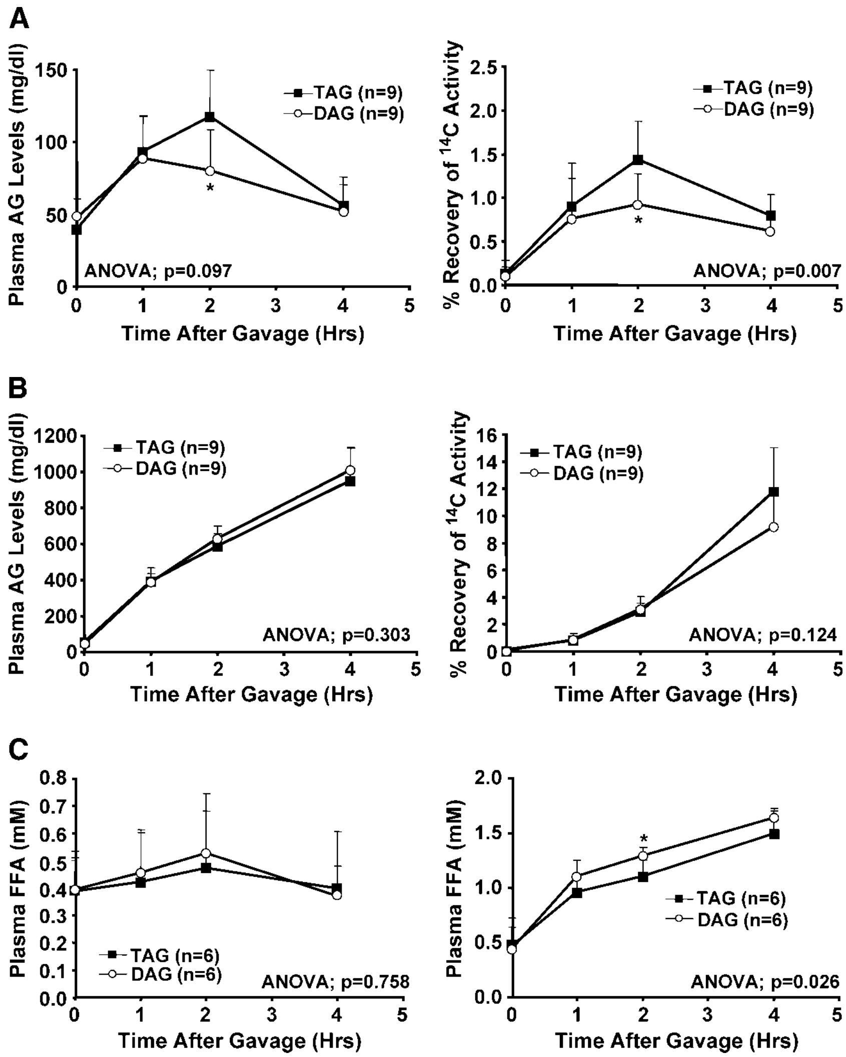

Fig. 3. Plasma levels of AGs and FAs after gavage of radiolabeled DAG and TAG emulsions with and without Triton WR1339. A: Plasma levels of AGs (left panel; means ± SD, mg/dl) and ^{14}C-radiolabeled plasma lipids (right panel; means ± SD, percentage of injected radioactivity recovered) over 4 h after gavage of either TAG or DAG ^{14}C-radiolabeled emulsions without concomitant Triton WR1339 injection. B: Plasma levels of AGs and ^{14}C-radiolabeled plasma lipids over 4 h after gavage of either TAG or DAG emulsion with concomitant Triton WR1339 injection. C: Plasma FA levels over 4 h after gavage of each emulsion without (left panel) or with (right panel) concomitant injection of Triton WR1339. Data represent means ± SD, and the asterisks denote statistically significant differences between TAG and DAG groups at each individual time point ($P < 0.05$, assessed by Student's *t*-test). Repeated two-way ANOVA was performed on the overall data set, and *P* values are indicated.

The tissue distribution of each emulsion was determined immediately after the final blood sample was obtained, 30 min after bolus injection. Tissue delivery was expressed as a percentage of total radioactivity recovered (**Fig. 6A**). Significantly more DAG emulsion particles were taken up by liver by 30 min (TAG vs. DAG: 22.4 ± 2.8% vs. 48.3 ± 2.3%), whereas significantly more TAG particles were found in spleen, kidney, and muscle.

Tissue distribution of FA derived from each emulsion was also determined at 5 min after bolus injection of either 20% TAG or DAG emulsions radiolabeled with [^{14}C]TO or [^{14}C]DO, respectively. The patterns of tissue distribution were similar to those seen for the whole emulsion particle, with particularly predominant uptake of [^{14}C]DO in the liver (TAG vs. DAG: 27.6 ± 3.2% vs. 41.8 ± 4.2%), indicating that both whole particles and FAs derived from DAG emulsion were taken up preferentially by the liver compared with TAG (Fig. 6B).

To investigate the role of particle uptake further, we conducted separate experiments on the clearance of emulsions radiolabeled with [^{3}H]CEt after bolus injection into apoE-deficient mice. The disappearance of AGs derived from the DAG emulsion in apoE-deficient mice was faster than that from the TAG emulsion (**Fig. 7A**); this pattern was similar to the results obtained in normal mice (Fig. 5A). By contrast, the removal rate of whole DAG

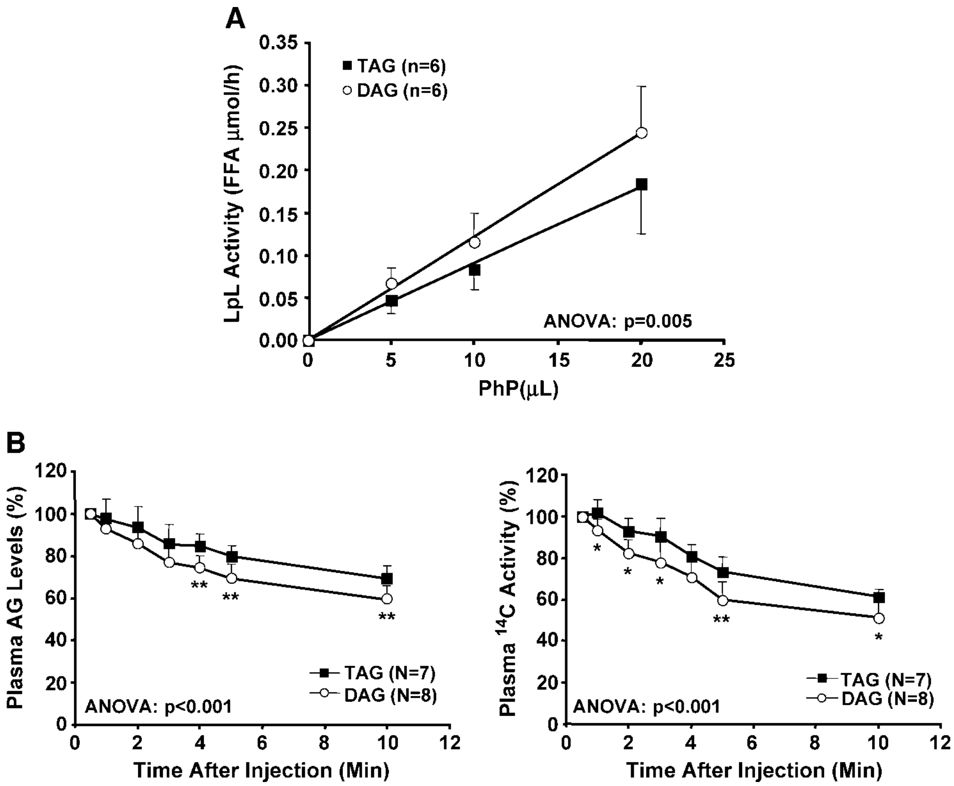

Fig. 4. Metabolic characteristics of chylomicron isolated from mice gavaged with DAG or TAG emulsions in the presence of Triton WR1339. A: Chylomicrons were isolated by ultracentrifugation at 120 min after gavage of either radiolabeled DAG or TAG emulsions concomitant with injection of Triton WR1339. The chylomicrons were incubated with increasing amounts of postheparin mouse plasma, and the release of [^{14}C]FA was used to determine the rate of lipolysis. Although there were no significant differences at each individual quantity of postheparinized plasma (PhP) as assessed by Student's *t*-test, repeated two-way ANOVA showed a significant difference between TAG and DAG for the overall data set ($P = 0.005$). B: Chylomicrons isolated as described above were injected intravenously into mice, and blood levels of AGs and radiolabeled emulsion lipids were determined over the next 10 min. In both panels, the data are presented as means ± SD of the levels present at 0.5 min. The asterisks denote statistically significant differences between TAG and DAG groups at each individual time point (** $P < 0.001$, * $P < 0.05$, assessed by Student's *t*-test). Repeated two-way ANOVA was performed on the overall data set, and *P* values are indicated.

particles in apoE-deficient mice was almost identical to that of TAG (Fig. 7B). Thus, the fractional catabolic rates for TAG and DAG particles in apoE-deficient mice were comparable (1.38 ± 0.26 and 2.03 ± 0.33 pool/h, respectively) and similar to the data obtained for whole TAG emulsion particles in normal mice (2.53 ± 1.1 pool/h).

Hydrolysis rates of emulsions using PhP in vitro assay

More rapid removal of AGs from DAG emulsions suggested better hydrolysis of the emulsion lipids by LPL. Therefore, we examined the in vitro hydrolysis rates of lipids in TAG and DAG emulsions by human PhP. The results demonstrate that the rate of lipolysis of lipids in the DAG emulsion was 7-fold higher than that in the TAG emulsion (TAG vs. DAG: 4.9 vs. 37.1 μmol FA/ml PhP/h) (**Fig. 8**).

Changes in plasma TG and FA in response to prolonged infusions of emulsion

We next infused DAG and TAG emulsions into normal mice for 6 h to investigate the impact of prolonged exposure to each AG on hepatic TG accumulation and the secretion of apoB and TG. We initially carried out preliminary studies to determine the optimal concentration of emulsion and the time course of change in plasma TG and FAs in response to the infusions of 5–20% TAG and DAG. After 6 h, the 5% DAG infusion did not increase plasma TG as much as the 5% TAG infusion, although both increased plasma TG linearly in response to increasing concentrations of infused emulsion (**Fig. 9**, left panel). On the other hand, at higher concentrations, the DAG infusion increased plasma FA more than the TAG infusion, suggesting that the more efficient generation of FAs by hydrolysis of DAG exceeded the capacity to internalize FAs into tissues (Fig. 9, right panel). Based on these studies, we selected 5% emulsions to examine the effects of emulsions on hepatic lipid and apoB metabolism. At the end of 6 h infusions with 5% DAG or 5% TAG, plasma TG concentrations were 43.3 ± 10.1 mg/l with DAG and 185.8 ± 39.1 mg/l with TAG, whereas plasma FA concentrations were 0.68 ± 0.25 mM after TAG and 0.59 ± 0.20 mM after DAG infusion.

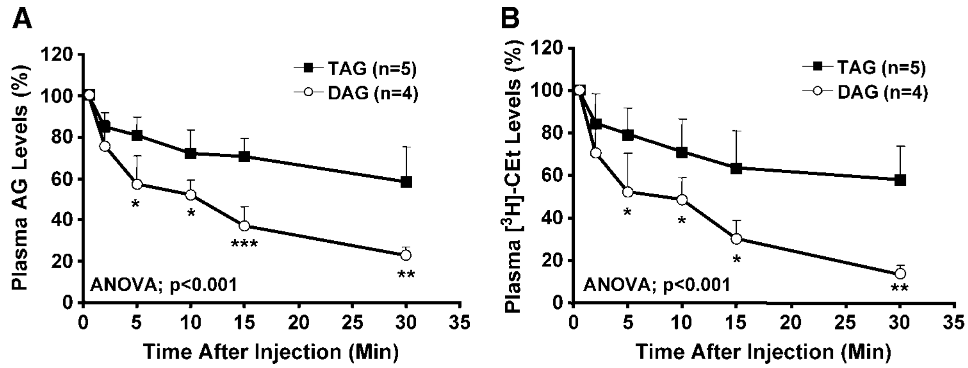

Fig. 5. Blood clearance of lipids and particles derived from DAG and TAG emulsions after intravenous bolus injections in normal mice. Mice received 100 μl of emulsion containing 20 mg of either TAG or DAG by bolus injection via the femoral vein. Clearance of lipids (A) and emulsion particles (B) from blood was assessed by measuring the levels of AGs and [^{3}H]cholesterol oleoyl ether ([^{3}H]CEt), respectively, in blood over a 30 min period. Data represent means ± SD, percentage of the value at 0.5 min after injection. The asterisks denote statistically significant differences between TAG and DAG groups at each individual time point (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, assessed by Student's *t*-test). Repeated two-way ANOVA was performed on the overall data, and *P* values are indicated.

After choosing 5% emulsions, we determined plasma TG and FA levels during 6 h infusions of TAG, DAG, and saline. Plasma TG and FAs were measured at baseline and at 1, 2, 3, and 6 h after the start of each infusion. Plasma FA levels increased in all three groups of mice during the first hour of infusion, suggesting the effects of both stress and emulsion delivery (**Fig. 10A**). However, in mice infused with saline, FA levels decreased rapidly toward the baseline, reaching a plateau of ~0.5 mM. In mice infused with TAG or DAG emulsions, FA levels also decreased gradually toward baseline but maintained higher levels than saline throughout the 6 h infusions. There was no significant difference in the levels of FAs during infusions of TAG or DAG. By contrast, TAG infusion induced a significant increase in plasma TG over time compared with saline and DAG infusions (Fig. 10B).

Hepatic uptake of FAs and accumulation of TG in response to prolonged infusions of emulsion

The discordant effects of TAG and DAG infusions on plasma lipid levels suggested different delivery of emulsion lipids into, and accumulation of TG by, the liver during each infusion. To estimate the actual amounts of FA delivered to the liver during the 6 h infusions, we injected a bolus of either TAG or DAG emulsion, radiolabeled with [^{14}C]TO or [^{14}C]DO, through the jugular vein after 4.5 h of infusion of unlabeled 5% TAG or DAG emulsion, respectively. The quantity of the tracer injected was equivalent to the amount of unlabeled emulsion delivered during 1 min of the infusion of 5% TAG or DAG emulsion. The results demonstrated that 32.6% of the TAG-derived FAs and 49.4% of the DAG-derived FAs had been taken up by liver at 5 min after injection of each tracer (**Fig. 11**).

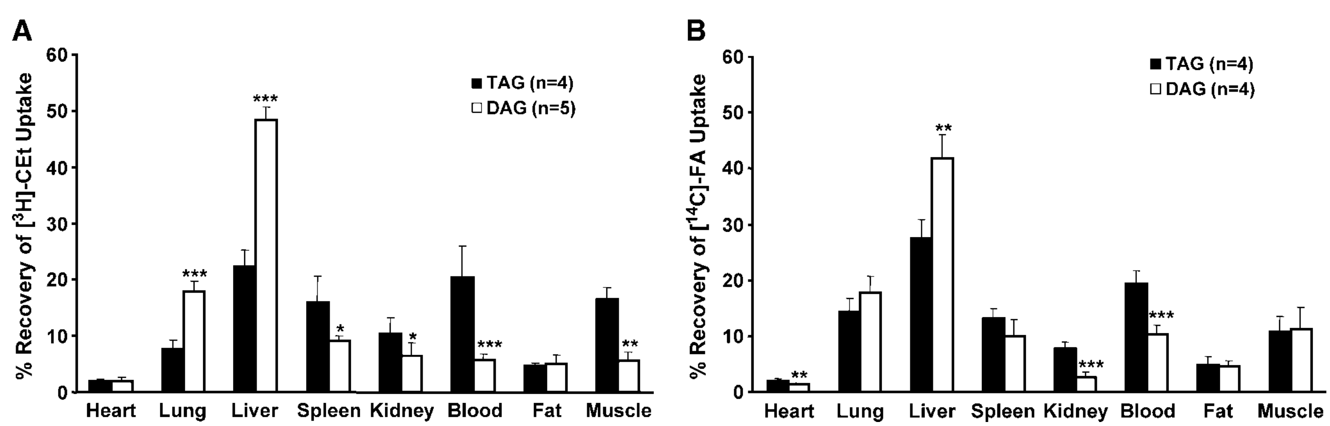

Fig. 6. Tissue distribution of particles and FAs derived from TAG and DAG emulsions after intravenous bolus injections in normal mice. Tissue distribution of particles (assessed by [^{3}H]CEt; A) and FAs (assessed by [^{14}C]TO or [^{14}C]DO; B) is expressed as percentage of total radioactivity recovered from organ and tissues. Total fat and muscle masses were calculated as 15% and 42% of body weight, respectively. Data represent means ± SD (4–5 mice per group), and asterisks denote statistically significant differences between TAG and DAG groups (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

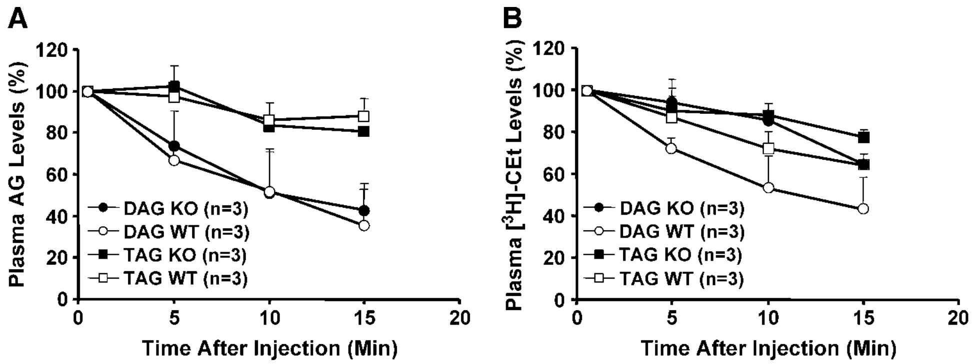

Fig. 7. Blood clearance of lipids and particles derived from DAG and TAG emulsions after intravenous bolus injections in apolipoprotein E (apoE)-deficient mice. Mice received 100 μl of emulsion containing 20 mg of either TAG or DAG by bolus injection via the femoral vein. Clearance of lipids (A) and emulsion particles (B) from blood was assessed by measuring the level of AGs and [^{3}H]CEt in blood after emulsion injection. Data represent mean percentages of the value at 0.5 min after injection, and error bars indicate SD. KO, knockout; WT, wild type.

These data are comparable to the data obtained from the bolus injection, in which 27.6% of TAG-derived FAs and 41.8% of DAG-derived FAs were taken up by liver. More DAG-derived FAs were also delivered to fat and muscle, whereas more TAG-derived FAs were found in lung, spleen, and blood.

Next, we measured liver TG content at the end of the 6 h infusions (**Fig. 12A**). The results demonstrate that the livers from mice infused with TAG and DAG had much higher TG content than those from mice infused with saline (TAG, 348.7 ± 10.7 μg/mg protein; DAG, 341.1 ± 28.0 μg/mg protein; saline, 131.2 ± 10.8 μg/mg protein). There was no significant difference in TG content between livers infused with TAG or DAG. Of note, TLC assay revealed that the predominant lipid in the liver of mice infused with DAG for 6 h was TAG. Furthermore, DAG content in livers were negligible, and there were no differences in DAG levels among the mice receiving DAG, TAG, or saline infusions. These results suggested that DAG

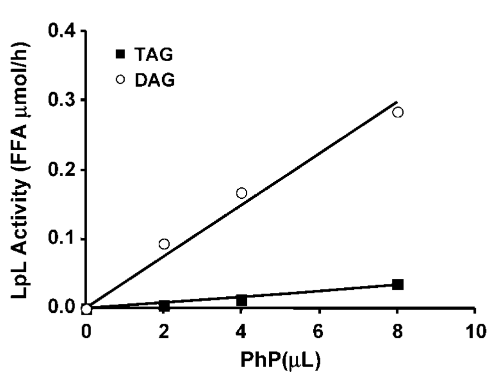

Fig. 8. In vitro hydrolysis of TAG and DAG emulsions by human LPL. TAG or DAG emulsions, labeled with [^{14}C]TO or [^{14}C]DO, respectively, were incubated with increasing volumes of PhP at 25°C for 1 h. The hydrolysis rate was determined by the amount of [^{14}C]FAs released from the emulsion. Experiments were done in triplicate.

infused intravenously was quickly metabolized into FAs, either in the plasma or in the liver (after emulsion particle uptake), and then resynthesized to TAG in liver.

Secretion of hepatic apoB and endogenous TG in response to prolonged infusions of emulsion

The finding that both DAG- and TAG-infused mice had similar increases in liver TG compared with saline-infused mice was surprising considering the increased uptake of FAs and emulsion particles during DAG infusions. To investigate this finding further, we determined the effect of TAG and DAG infusions on VLDL secretion rates, examining both TG and apoB secretion after injection of Triton WR1339 at the end of each 6 h infusion (20). At the end of 6 h infusions, plasma TG levels were significantly higher in the mice infused with TAG than in the mice infused with either saline or DAG (plasma TG on TAG, 173.4 ± 31.4 mg/dl; plasma TG on DAG, 40.6 ± 19.71 mg/dl; plasma TG on saline, 21.2 ± 7.7 mg/dl). After injection of Triton WR1339, plasma TG levels increased steadily over the next 120 min in all groups; the rate of increase in plasma TG was significantly greater in mice infused with DAG versus mice infused with TAG or saline, although the TAG infusion increased the rate of increase in plasma TG significantly greater than saline infusion (Fig. 12B). The actual rates of increase in plasma TG between 30 and 120 min after Triton WR1339 injection were 3.13 ± 0.42, 2.23 ± 0.36, and 1.84 ± 0.26 mg TG/dl/h for DAG, TAG, and saline, respectively (DAG vs. saline, $P < 0.001$; TAG vs. saline, $P < 0.05$; DAG vs. TAG, $P < 0.01$). Concomitantly, the secretion of newly synthesized apoB-100 during 120 min after Triton WR1339 injection was increased in both the TAG and DAG groups compared with the saline group (178 ± 64%, 169 ± 45%, and 100 ± 14%, respectively; DAG vs. saline, $P < 0.05$; TAG vs. saline, $P < 0.05$) (Fig. 12C). Similar patterns of the appearance of newly synthesized apoB-48 in plasma were also observed (137 ± 41%, 168 ± 17%, and 100 ± 9%, respectively). There was no significant difference in the secretion of

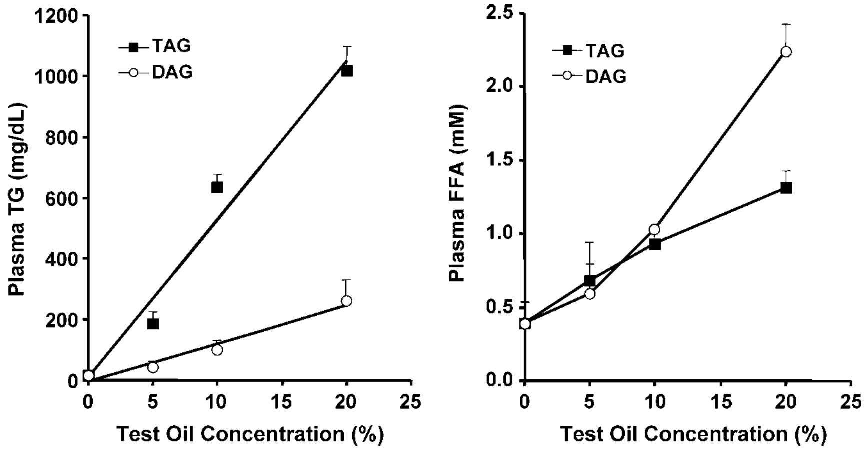

Fig. 9. Dose response of plasma triglyceride (TG) and FAs after 6 h infusions of TAG and DAG emulsions. Plasma TG (left panel) and FA (right panel) levels are shown after 6 h infusions of TAG and DAG emulsions at various concentrations (5, 10, and 20%). Data represent means ± SD. n = 2–10 mice per group at each test oil concentration.

apoB-100 and apoB-48 between mice infused with TAG and DAG. These findings, indicating higher TG secretion but comparable apoB secretion between TAG- and DAG-infused groups, suggested that the livers of mice infused with DAG secreted larger VLDLs than the livers of mice infused with TAG.

Expression of hepatic mRNA involved with β-oxidation in response to emulsion infusions

To determine whether the increased FA delivery to the livers of mice infused with DAG induced β-oxidation, we examined the hepatic expression of mRNAs for acyl-coenzyme A oxidase (AOX), carnitine palmitoyltransferase-1 (CPT-1), and peroxisome proliferator-activated receptor α (PPARα). The mRNA levels of the mice infused for 6 h with either TAG or DAG were expressed relative to normalized levels from mice infused with saline (**Fig. 13**). The results demonstrate that there was a small but significant increase in the expression of AOX in mice infused with the DAG emulsion. However, there were no differential effects of DAG or TAG versus saline on the expression of PPARα or CPT-1.

DISCUSSION

Intravenously administered lipid emulsions are reported to be metabolized by a pathway to TG-rich lipoproteins; they acquire apolipoproteins from circulating lipoproteins and are taken up, mainly by liver, after hydrolysis by LPL (10, 11). A large body of studies has been conducted to investigate the roles of specific core lipid FAs in blood clearance and tissue delivery (12–16). However, the metabolism of intravenous lipid emulsions primarily composed of different AG structures has been poorly defined. Recently 1,3-DAG, in contrast to TAG, was reported to enter distinct metabolic pathways in the gastrointestinal tract, where the main digestive product of 1,3-DAG is 1-MAG (or 3-MAG), an AG that is poorly reesterified into TAG in the intestinal mucosa (2, 7, 9). In addition, DAG is reported to exert different physicochemical properties with respect to the oil-water interface and emulsification compared with TAG (19); this might affect the interaction of DAG emulsions with LPL or cellular endocytic pathways. With these findings in mind, we initiated two series of studies. First, we examined, in vivo, the

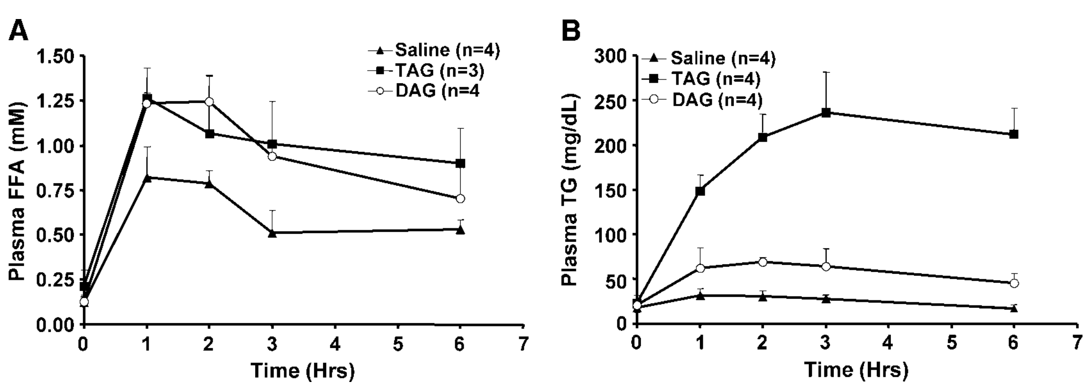

Fig. 10. Changes in plasma FA and TG levels during infusions of 5% TAG, DAG, and saline. Time courses for changes in plasma FA (A) and TG (B) levels during infusion of 5% TAG and DAG emulsions, as well as saline, are shown. Data represent means ± SD.

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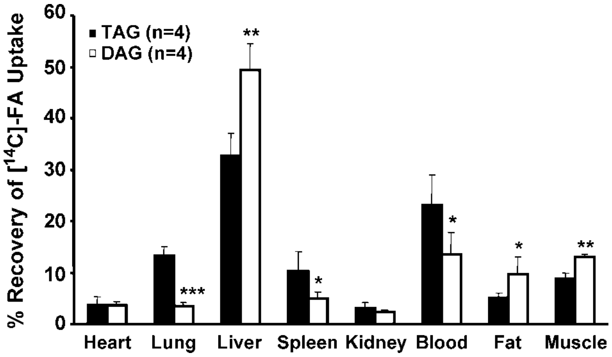

Fig. 11. Tissue distribution of FA derived from TAG and DAG emulsions during 6 h infusions in normal mice. To investigate the relative delivery of FAs to the liver during prolonged infusions of TAG and DAG, 0.125% TAG or DAG radiolabeled with [^{14}C]TO or [^{14}C]DO was injected rapidly through the jugular vein after 4.5 h infusions with unlabeled 5% emulsions of TAG or DAG, respectively. The quantity of tracer injected approximated the amount of unlabeled emulsion delivered during 1 min of the 4.5 h infusion. Five minutes after bolus injections, mice were euthanized, and organs and tissues were collected. Tissue distribution was assessed as described in Materials and Methods and in the legend to Figure 6. Data represent means ± SD, and asterisks denote statistically significant differences between the TAG and DAG groups (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

appearance and catabolism of chylomicrons in mice gavaged with either DAG or TAG emulsions. Second, we studied, in detail, the blood clearance of both orally and intravenously administered DAG and TAG emulsions, the tissue uptake and distribution of intravenously administered DAG and TAG emulsions, and, finally, the effects of each emulsion on VLDL secretion from the liver.

Our initial studies focused on the proposal, based on published studies (2, 7, 8), that 1,3-DAG ingestion is associated with lower postprandial TG levels because of inefficient incorporation of FAs into 1-MAG or glycerol in enterocytes. We used Triton WR1339 to examine the assembly and secretion of chylomicrons after ingestion of DAG or TAG; although we did demonstrate slightly less TAG in chylomicrons in the mice gavaged with DAG emulsion, there was more MAG and DAG. Overall, we found no evidence of defective incorporation of orally ingested DAG-FAs into chylomicrons. By contrast, our results indicate that the reduced postprandial TG levels are attributable to more efficient interaction with, and hydrolysis by, LPL of DAG-generated chylomicrons. Furthermore, our data suggest that the more efficient lipolysis results from very modest increases in chylomicron MAG and 1,3-DAG.

We next focused on the metabolism of intravenously administered DAG and TAG emulsions. Tracer studies after bolus injections of radiolabeled emulsions revealed that both core lipids and whole particles derived from DAG emulsions were cleared faster from blood than those from TAG emulsions. The more rapid clearance of DAG was consistent with the observation that during 6 h infusions of 5% DAG and 5% TAG emulsions, TAG increased plasma TG significantly but the DAG emulsion had no effect. Measures of tissue FA accumulation, after bolus injections of DAG and TAG, and after several hours of DAG and TAG infusions, demonstrated the preferential hepatic uptake of FAs and emulsion particles derived from DAG. Thus, independent of size and overall FA composition, DAG emulsions were removed from the plasma more efficiently than TAG emulsions.

A major contributor to the clearance of TG-rich lipoproteins from plasma is LPL. We demonstrated that, in vitro, DAG emulsions were better substrates for LPL-mediated lipolysis. These results were in concert with the ex vivo and donor-recipient mouse studies performed with chylomicrons described above. The efficient hydrolysis of DAG by LPL is likely attributable to its distinct physicochemical properties. More DAG molecules can be partitioned at the surface of emulsions as a result of their

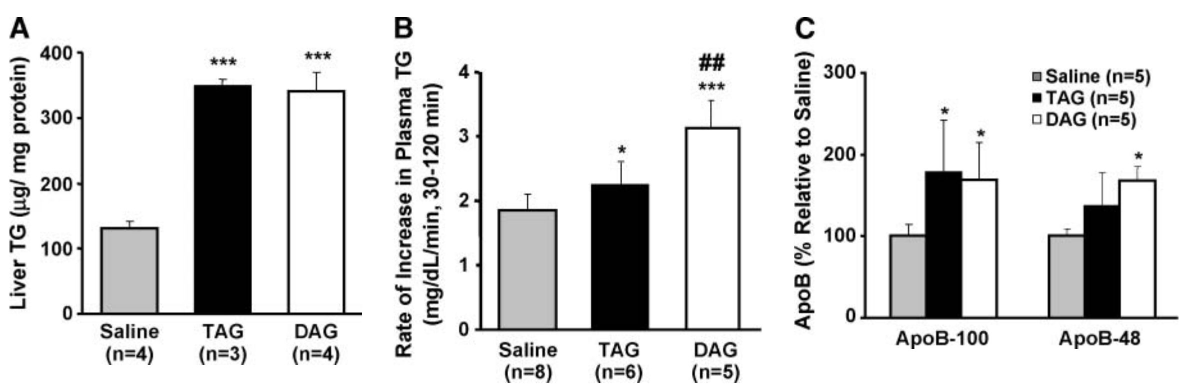

Fig. 12. Hepatic response to 6 h infusions of TAG, DAG, and saline. A: Liver TG of mice infused with saline, TAG, or DAG for 6 h. Data represent means ± SD, and asterisks denote statistically significant differences from saline-infused mice (*** $P < 0.05$). B: Secretion of endogenous TG determined by the increment of TG from 30 to 120 min after injection of Triton WR1339. Data represent means ± SD, and asterisks denote statistically significant differences (* $P < 0.05$, *** $P < 0.001$, compared with saline-infused mice; $^{\#\#}$ $P < 0.01$, compared with TAG). C: Secretion of newly synthesized apoB-100 and apoB-48 into plasma was estimated from the incorporation of [^{35}S]methionine into plasma apoB at 120 min after injection of Triton WR1339 and [^{35}S]methionine. Data represent means ± SD, and asterisks denote statistically significant differences (* $P < 0.05$).

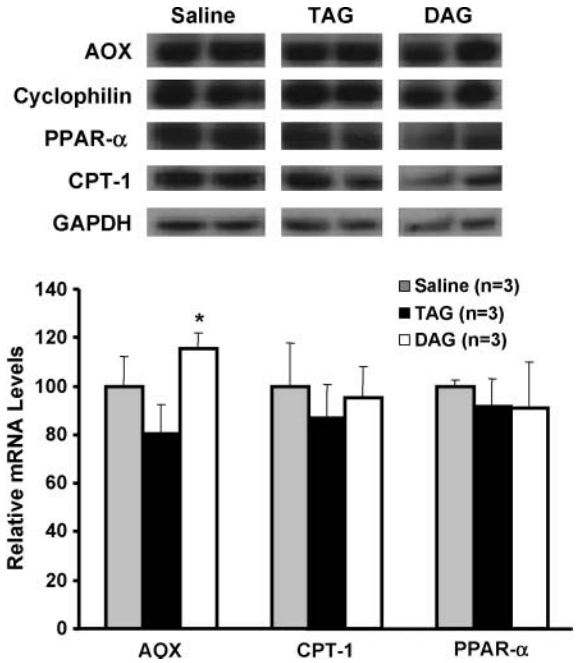

Fig. 13. Expression of hepatic mRNA involved with β-oxidation after 6 h infusions of TAG, DAG, and saline. Expression of mRNA involved with β-oxidation was measured with the RNase protection assay. Radioactivity of target bands [acyl-coenzyme A oxidase (AOX), peroxisome proliferator-activated receptor α (PPARα), and carnitine palmitoyltransferase-1 (CPT-1)] was normalized to the internal reference (cyclophilin or GAPDH). mRNA levels derived from mice infused with saline were averaged and expressed as 100%. mRNA levels derived from infusions of either TAG or DAG emulsions were expressed relative to data from saline-treated mice. Data represent means ± SD, and asterisks denote statistically significant differences (* $P < 0.01$, compared with TAG).

hydrophilicity, enabling LPL to interact with DAG easily compared with TAG molecules. Indeed, we have preliminary data that the addition of 1,3-DAG to TAG emulsions leads to better LPL-mediated lipolysis of the TAG (personal communication).

Our cholesteryl ether studies indicated that there was also better endocytic uptake of DAG particles and that this uptake was apoE-dependent. Thus, we observed that although the clearance of core AGs derived from DAG emulsion was faster than that from TAG emulsion in apoE-deficient mice (similar to results obtained in normal mice), blood clearance of whole DAG particles in apoE-deficient mice was the same as that of TAG emulsion particles. Overall, we believe that our results indicate that the rapid clearance of the DAG emulsion is attributable to the efficient hydrolysis of the core lipids by LPL, which facilitates conversion of the emulsion to remnant-like particles that are taken up by the liver through an apoE-dependent pathway. Because we did not study interactions between DAG and TAG emulsions and HL, we cannot rule out a greater affinity of a DAG-derived remnant for HL. However, the fact that the differences in particle clearance between DAG- and TAG-derived remnants were abrogated by the absence of apoE suggests the neither HL-mediated lipolysis of remnant AGs, nor HL acting as a remnant ligand, played a significant role in the differences observed in normal mice. On the other hand, we cannot rule out a role for HL, either via lipolysis or as a ligand, as an "assistant" to apoE-mediated uptake of emulsion-derived remnants (34). Of interest, it has been well demonstrated that surface lipids of emulsions alter the affinity of apolipoproteins (35, 36), and one study revealed a distinct difference between phospholipids coating DAG emulsions and those coating TAG emulsions (37), raising the possibility of differential apolipoprotein acquisition in the blood stream.

Our finding that DAG emulsions delivered more FAs to the liver than TAG emulsions suggested that there might be different effects of each on hepatic lipid metabolism, particularly hepatic TG accumulation and the secretion of VLDL TG and apoB. The amounts of FAs infused with TAG and DAG emulsions for 6 h were similar: 43.1 and 41.0 mg, respectively. However, as noted above, FA uptake by the liver was significantly greater during the infusion of DAG. Surprisingly, despite increased delivery of FA to the liver during the infusion of DAG, hepatic TG was the same with DAG and TAG; both infusions were associated with greater liver TG compared with saline-infused mice. Studies of VLDL TG and apoB secretion provided the basis for the apparent dissociation between FA delivery and TG accumulation in the liver. Thus, mice infused with either DAG or TAG emulsions had similar increases in apoB secretion. However, secretion of VLDL TG was significantly greater after DAG infusion. These findings suggested that larger particles of VLDL were secreted from livers of mice infused with DAG emulsion compared with mice infused with TAG emulsion. These results are consistent with our previous data demonstrating that TAG emulsion induced both hepatic apoB and TG secretion compared with saline (20). These data extend our prior work and indicate that the liver, in response to an increased influx of FAs, can both increase the number of VLDL secreted and "load" additional TG onto each apoB particle. Furthermore, the assembly and secretion of larger VLDL particles after DAG emulsion infusion may have contributed to the maintenance of low levels of plasma TG during DAG infusion, because larger lipoproteins are better substrates for LPL-mediated lipolysis. Of course, the lower ambient TG levels in mice infused with DAG emulsion might also present less competition for LPL, allowing better lipolysis of newly secreted VLDL (38).

Induction in hepatic β-oxidation of FA would be another way that hepatic TG levels would be modulated in the face of more rapid and greater delivery of FAs by DAG. Therefore, we determined the expression levels of hepatic mRNA of key genes involved in β-oxidation in each group of mice. There were no systematic differences between groups in the expression of PPARα, CPT-1, or AOX, indicating that infusion with DAG emulsion for 6 h did not alter hepatic β-oxidation compared with that in mice infused with TAG and saline.

In conclusion, our studies demonstrate the following. *1*) DAG and TAG emulsions provided orally are incorporated into chylomicrons with equal efficiency. *2*) Chylomicrons generated by oral ingestion of DAG are better substrates for LPL and are cleared more efficiently from plasma than chylomicrons generated from TAG. *3*) DAG administered intravenously is cleared more efficiently than TAG; LPL and apoE endocytosis play key roles in this process. *4*) Despite more efficient and more rapid delivery of FAs to the liver by DAG, hepatic TG contents are the same after infusions of DAG and TAG because the liver secretes larger VLDL particles with more TG per particle after DAG infusion; the larger VLDL particles secreted in response to DAG are probably better substrates for subsequent hydrolysis by LPL. *5*) The more efficient metabolism of DAG emulsions could be useful when parenteral nutrition is necessary in patients with preexisting hypertriglyceridemia. We note, however, that a recent study, in which DAG oil consumption appeared to reduce plasma TG levels in a patient homozygous for LPL deficiency, leaves open other mechanisms whereby DAG oil may be associated with reduced postprandial TG concentrations (39).

The authors thank Mr. Yoshinobu Nakajima of the Kao Corporation (Tokyo, Japan) for his technical advice and efforts to prepare and characterize the DAG emulsion. This work was supported by the following grants: National Institutes of Health Grants R01 HL-73030 and R01 HL-55638 and a grant from the Kao Corporation.

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