

Intestinal DGAT1 deficiency reduces postprandial triglyceride and retinyl ester excursions by inhibiting chylomicron secretion and delaying gastric emptying

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Abstract Acyl CoA:diacylglycerol acyltransferase (DGAT) 1 catalyzes the final step of triglyceride (TG) synthesis. We show that acute administration of a DGAT1 inhibitor (DGAT1i) by oral gavage or genetic deletion of intestinal *Dgat1* (intestine-*Dgat1*^{-/-}) markedly reduced postprandial plasma TG and retinyl ester excursions by inhibiting chylomicron secretion in mice. Loss of DGAT1 activity did not affect the efficiency of retinol esterification, but it did reduce TG and retinoid accumulation in the small intestine. In contrast, inhibition of microsomal triglyceride transfer protein (MTP) reduced chylomicron secretion after oral fat/retinol loads, but with accumulation of dietary TG and retinoids in the small intestine. Lack of intestinal accumulation of TG and retinoids in DGAT1i-treated or intestine-*Dgat1*^{-/-} mice resulted, in part, from delayed gastric emptying associated with increased plasma levels of glucagon-like peptide (GLP)-1. However, neither bypassing the stomach through duodenal oil injection nor inhibiting the receptor for GLP-1 normalized postprandial TG or retinyl ester excursions in the absence of DGAT1 activity. **In summary, intestinal DGAT1 inhibition or deficiency acutely delayed gastric emptying and inhibited chylomicron secretion; however, the latter occurred when gastric emptying was normal or when lipid was administered directly into the small intestine. Long-term hepatic retinoid metabolism was not impacted by DGAT1 inhibition.**—Ables, G. P., K. J. Z. Yang, S. Vogel, A. Hernandez-Ono, S. Yu, J. J. Yuen, S. Birtles, L. K. Buckett, A. V. Turnbull, I. J. Goldberg, W. S. Blaner, L.-S. Huang, and H. N. Ginsberg. **Intestinal DGAT1 deficiency reduces postprandial triglyceride and retinyl ester excursions by inhibiting chylomicron secretion and delaying gastric emptying.** *J. Lipid Res.* 2012. 53: 2364–2379.

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Elevated levels of plasma triglyceride (TG), whether derived from secretion of very low density lipoproteins (VLDL) or chylomicrons, are major components of the metabolic syndrome and are important risk factors in the development of atherosclerotic cardiovascular disease (1). The final step in TG biosynthesis is the addition of fatty acyl-CoA (CoA) to diacylglycerol, which is catalyzed by one of two acyl CoA:diacylglycerol acyltransferases (DGAT). DGAT1 and DGAT2 are encoded by two genes belonging to different families that do not share significant sequence homology (2–5). Both genes are ubiquitously expressed but with highest expression levels found in tissues active in TG synthesis, such as adipose tissue, small intestine, liver, and mammary gland. Mice lacking DGAT2 are severely lipopenic and die shortly after birth (6). Inhibition studies demonstrate a major role of DGAT2 in hepatic lipid homeostasis (7–9), although the role of DGAT2 on VLDL secretion is less clear (7, 9, 10). DGAT1-deficient (*Dgat1*^{-/-}) mice are viable, have modest reductions in tissue TG content, and have normal plasma TG levels (11). Again, effects of DGAT1 on VLDL secretion have varied (10, 12, 13). *Dgat1*^{-/-} mice are protected from diet-induced as well as some genetic forms of obesity through an increase in energy expenditure (11, 14). They also exhibit increased sensitivity to insulin and leptin and are protected from diet-induced insulin resistance (14, 15). Mice lacking DGAT1 also have significantly reduced postprandial excursions of TG and retinyl esters, i.e., their

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Abbreviations: DGAT1i, DGAT1 inhibitor; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; HPMC, hydroxypropylmethylcellulose; KO, knockout; LRAT, lecithin:retinol acyltransferase; MTP, microsomal triglyceride transfer protein; PVP, polyvinyl pyrrolidone; TG, triglyceride; WT, wild-type; WTD, Western-type diet.

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plasma levels over the course of several hours, after oral administration of oil and retinol (11, 16).

The phenotype of the *Dgat1*^{-/-} mice spurred significant interest in the development of DGAT1 inhibitors (reviewed in Ref. 17). Pharmacological inhibition of DGAT1 has been shown to reduce plasma TG excursions following oral lipid tolerance tests in rats and mice (18–22). This has been attributed to inhibition of DGAT1 in the gastrointestinal tract, where DGAT2 is expressed at much lower levels (16). DGAT1 inhibitors have also prevented weight gain or even contributed to weight loss in the setting of diet-induced obesity, although not in leptin-deficient rodent models (references within Ref. 17). In some but not all studies, DGAT1 inhibitors reduced food intake of mice on a high-fat diet (17). Because of this, it has been suggested that alterations in the secretion of gastrointestinal peptides affecting satiety and/or energy expenditure occur in response to DGAT1 inhibition in the face of high fat loads.

Because vitamin A is a fat-soluble dietary component, the process of vitamin A esterification to retinyl esters prior to exit from the intestine is closely associated with TG synthesis and chylomicron assembly and secretion. Although lecithin:retinol acyltransferase (LRAT) is established to be the major enzyme for esterification of vitamin A in most organs within the body (23), the intestine and skin also synthesize retinyl esters in an acyl-CoA-dependent manner, involving an acyl-CoA:retinol acyltransferase (ARAT). DGAT1 exhibits ARAT activity in vitro (24, 25) and acts physiologically as an ARAT in the murine intestine (23, 26) and skin (27). Consequently, there is considerable interest in understanding the effects that DGAT1 inhibitors may have on retinoid homeostasis.

We explored, therefore, the effects of both acute pharmacologic inhibition of DGAT1 and genetic deletion of intestinal *Dgat1* (intestine-*Dgat1*^{-/-}) on the absorption of TG and retinol in the small intestine and chylomicron secretion as measured by the appearance of orally administered TG and retinol in the plasma of mice treated with inhibitors of lipoprotein removal (e.g., Triton WR1330 or P-407). We also examined the link between DGAT1 inhibition, gastric emptying, and the secretion of glucagon-like peptide (GLP)-1, a gut hormone implicated in the regulation of a number of metabolic activities, including gastric emptying (28). We show that both acute pharmacologic inhibition of DGAT1 and genetic deletion of intestinal *Dgat1* result in delayed gastric emptying. However, we also demonstrate that the absence of DGAT1 activity directly reduces post fat load plasma TG and retinoid excursions by inhibiting the secretion of chylomicrons. Importantly, chronic pharmacologic inhibition of DGAT1 does not alter total body retinoid homeostasis.

MATERIALS AND METHODS

Mice and diets

All animal protocols were in compliance with accepted standards of animal care and approved by the Columbia University Institutional Animal Care and Use Committee. Age-matched

male mice (8–12 weeks of age) were used for all experiments unless otherwise indicated. Wild-type (WT) C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME).

Mice heterozygous for *Dgat1*^{flox/flox} (i.e., *Dgat1*^{flox/+}) (29) were generously provided by Dr. Robert Farese, Jr., at the Gladstone Institute. The *flox* allele (>150 bp) was distinguished from the WT allele (150 bp) using gene sequences flanking one of the LoxP sites. The forward primer sequence is 5'-CTGTGGCAGAGCTTCTGC and the reverse primer sequence is 5'-AGCATAGGCTTGTAGAAGTGT. *Dgat1*^{flox/flox} mice were subsequently generated and crossed to mice expressing Cre-recombinase under the control of the villin (*Vil*) gene promoter (Jackson Laboratory), leading to the specific deletion of the *Dgat1* gene in intestinal epithelial cells. The identification of the *Vil*-Cre allele was based on the method described in the Jackson Laboratory web site. In all experiments, intestine-*Dgat1*^{-/-} mice (*Vil*-Cre/*Dgat1*^{flox/flox}) were compared with control (*Dgat1*^{flox/flox}) littermates.

All mice were housed in a barrier facility under a 12 h light/12 h dark cycle. Mice had ad libitum access to either chow diet (5053 PicoLab Rodent Diet 20; Purina Mills) or the Western-type diet (WTD) (WTD 88137; Teklad Premier Laboratory Diets, Madison, WI), providing 42% of calories as fat (polyunsaturated/saturated = 0.07) and containing high sucrose levels (29% of calories) and 0.15% (w/w) cholesterol.

Determination of DGAT inhibitory activity of compound 2, a DGAT1 inhibitor

The DGAT1 inhibitor (DGAT1i) used in these experiments was reported previously as compound 2 (18). It is a potent and selective small-molecule inhibitor of human DGAT1 (15 nM) that has also been shown to potently inhibit mouse microsomal DGAT1 activity (11 nM) (21). The DGAT inhibitory activity of compound 2 was assessed using recombinant human DGAT1 and mouse liver microsomes. Recombinant human DGAT1 was prepared according to the method of Cases et al. (2). Mouse liver microsomes were prepared and DGAT activity assayed by a modification of the method described by Coleman (30). Compound 2 in 1% DMSO was incubated with 4 µg/ml membrane protein, 5 mM MgCl₂, and 100 µM 1,2 dioleoyl-*sn*-glycerol (in 10% acetone) in a total assay volume of 200 µl in a 96-well plate. The reaction was started by adding [¹⁴C] oleoyl CoA (30 µM) and incubated at room temperature for 30 min. The reaction was stopped by adding 200 µl isopropanol:heptane (7:1, v/v). Radioactive triolein product was separated into the organic phase by adding heptane (300 µl) and 0.1 M carbonate buffer pH 9.5 (100 µl). DGAT activity was quantified by counting aliquots of the upper heptane layer by liquid scintigraphy.

Acute treatment of mice with a DGAT1i

A stock solution of DGAT1i (5 mg/ml or 12.675 µmol/ml) was formulated in the vehicle, consisting of 0.5% w/v hydroxypropylmethylcellulose (HPMC) in 0.1% w/v Tween 80, for oral dosing. The suspensions were prepared the day before the start of the study and were stirred continuously at ambient temperature overnight prior to dosing to give adequate particle size reduction. The experiment began at 8 AM after removal of food.

Eight-week-old mice fed a chow diet or 14-week-old mice fed 6 weeks of WTD were orally gavaged with vehicle or DGAT1i (5 mg/kg body weight unless otherwise indicated) upon food removal at 8 AM. For the initial pharmacokinetic analysis of DGAT1i, three doses (1 mg/kg, 5 mg/kg, and 50 mg/kg) were used. Mice were bled at various time points, and plasma DGAT1i concentration measured as described (18).

Postprandial plasma TG and retinoid excursions

Two hours after drug administration, mice were bled at time 0 and immediately gavaged with corn oil (200 µl) with or without

tracers to assess postprandial TG excursions. In some studies, the corn oil contained a physiological dose of retinol (6 µg) and 2×10^6 cpm of [^3H]retinol ([11, 12- ^3H (N)]retinol; Perkin Elmer). For postprandial TG excursion, mice were bled at 1, 2, 4, and 6 h after the fat challenge. Plasma TG levels were measured using Infinity Triglycerides Reagent (Fisher Scientific) as described previously (31). Plasma (20 µl) was assessed for [^3H]retinoid counts by liquid scintillation counting. Total plasma [^3H]retinoid counts (cpm) were then calculated based on the plasma volume of any given animal. Intestine-*Dgat1*^{-/-} mice underwent the same protocol without administration of DGAT1i or vehicle.

Determination of chylomicron secretion rates

Assessment of chylomicron secretion rates was performed as described previously (31). Animals were given the DGAT1i or HPMC vehicle starting at 8 AM. Two hours after drug administration, mice were bled (time 0), followed by an intravenous injection of 500 mg/kg Triton WR1339 (Sigma-Aldrich) in 0.9% NaCl and an oral gavage of 200 µl of corn oil containing 6×10^5 cpm [^3H]triolein ([9, 10- ^3H (N)]triolein; Perkin Elmer) as a tracer for newly formed chylomicrons. In other experiments, mice were gavaged with 200 µl of corn oil containing a physiological dose of retinol (6 µg) and 2×10^6 cpm of [^3H]retinol. Blood samples were collected 0, 30, 60, 90, 120, and 240 min after injection of Triton. Plasma clearance of TG-rich lipoproteins (chylomicrons or VLDL) is completely inhibited after injection of Triton WR1339 in mice under these conditions, and the accumulation of TG in plasma can be used to estimate rates of secretion of newly assembled chylomicrons and/or VLDL. The accumulation of either radiolabeled TG or radiolabeled retinoids in plasma after Triton WR1339 injection provides a specific estimate of the rate of secretion of newly assembled chylomicrons.

Determination of TG and retinol absorption

In some experiments, mice were given DGAT1i or HPMC vehicle starting at 8 AM. Two hours after drug administration, mice were gavaged with 200 µl of corn oil containing a physiological dose of retinol (6 µg) and 2×10^6 cpm of [^3H]retinol ([11, 12- ^3H (N)]retinol; Perkin Elmer). Blood samples were obtained over 6 h after gavage, at which time the mice were euthanized and tissues were collected for lipid measurement and assessment of tissue ^3H -cpm levels. The whole stomach was excised and opened, and the contents gently scraped into a scintillation vial. The ^3H -cpm present in the scrapings were considered to represent gastric contents. The stomach itself was frozen at -80°C until further analysis. Livers were weighed and aliquots were stored at -80°C for further analysis. The small intestine, starting immediately after the pyloric sphincter, was cut into four segments of 5 cm each and the rest, consisting of approximately 4–8 cm of the distal small intestine, was collected as the fifth segment. The colon was also removed. Upon collection of the small intestine sections and colon, each was cut longitudinally to expose the lumen, washed in PBS, gently blotted with tissue paper to remove debris, and then stored at -80°C until further analysis. Feces were collected from individual mice for ^3H -cpm analysis by placing each mouse in individual buckets. Intestine-*Dgat1*^{-/-} mice underwent the same protocol with and without administration of DGAT1i or vehicle.

To determine the reversibility of the effects of DGAT1 inhibition on chylomicron secretion and retinol absorption, mice were given DGAT1i or HPMC vehicle starting at 8 AM. Two hours after drug administration, mice were bled (marked as time 0), followed by an intraperitoneal injection of P-407 (1 g/kg body weight; BASA Corp.) in PBS before receiving an oil gavage as described above. P-407, like Triton WR1339, effectively blocks lipoprotein clearance but over a longer period of time (32). Blood samples were collected 2 h after drug administration and prior to

oil gavage (time 0) and at 1, 2, 4, 8, and 24 h after injection of P-407. Plasma aliquots were analyzed for TG and retinoid radioactivity as described above. For some experiments, plasma levels of [^3H]retinol and [^3H]retinyl esters were assessed following separation and collection of these retinoids by high performance liquid chromatography (HPLC) as described below for small intestine (26). Intestine-*Dgat1*^{-/-} mice underwent the same protocol without administration of DGAT1i or vehicle.

Acute treatment with dirlotapide, an inhibitor of microsomal triglyceride transfer protein

Mice were treated with dirlotapide (3 mg/kg body weight) to determine its effect on chylomicron secretion. Dirlotapide was dissolved in 0.67% of polyvinylpyrrolidone (PVP) (Kollidon 25; BASF) and 0.033% Aerosol OT (Aerosol OT-100; Cytec Industries) to a final concentration of 1 mg/ml concentration. The microsuspension was sonicated for 10 min and then stirred overnight. The formulation was diluted to a concentration of 0.3 mg/ml and administered at a dose of 10 ml/kg body weight. As a control for the dirlotapide, the PVP-containing vehicle was used. Male C57BL/6 mice (n = 5/group) were given HPMC, 5 mg/kg dose of DGAT1i, PVP, or 3 mg/kg dose of dirlotapide. Two hours after drug administration, mice were orally gavaged with [^3H]retinol-containing corn oil. Mice were bled before (time 0) and at various time points after fat challenge.

Determination of tissue TG content

Tissue samples weighing approximately 100–150 mg were homogenized in 2 ml of 1M NaCl solution. Twelve milliliters of chloroform/methanol (2:1, v/v) was added to each tube, which was vortexed vigorously and centrifuged at 1,000 g for 10 min at 4°C to separate phases. The lower chloroform phase containing triglycerides was transferred to a glass tube and allowed to evaporate under a gentle stream of N_2 until completely dry. This was followed by addition of 1 ml of 2% Triton X-100 in chloroform to each sample and removal of the chloroform under nitrogen gas. After drying, 1 ml of deionized water was added to each tube, and the samples were vortexed until completely dissolved. TG levels were measured using Infinity Triglycerides Reagent (Fisher Scientific) as described previously (31).

Analyses of retinol and retinyl ester by HPLC

Plasma and small-intestine fragments were processed for HPLC as previously described (26). Retinoids in plasma or tissue homogenate prepared in 1.0 ml PBS was extracted using chloroform:methanol (2:1, v/v). The chloroform extracts were evaporated under a gentle stream of nitrogen gas. The dried lipids were reconstituted in ethanol containing retinyl acetate as internal standard, vortexed, and then extracted twice with hexane. The hexane extracts were evaporated and reconstituted in benzene and analyzed for retinol and retinyl ester by reverse-phase HPLC (26). During the HPLC separation of retinol and retinyl ester, fractions containing retinol and retinyl ester were collected into scintillation vials and placed into a hood for evaporation overnight. After complete dryness was achieved, 10 ml of scintillation cocktail was added to each sample and ^3H -cpm levels were determined by liquid scintillation counting.

Determination of plasma GLP-1 levels

Total plasma GLP-1 (7-36 and 9-36 peptides) concentrations were measured using an ELISA kit (48-GP1HU-E01; Alpco Diagnostics, Salem, NH) following the manufacturer's protocol.

Chronic treatment with DGAT1i

C57BL/6 mice (n = 6 group) were treated with WTD containing 0.2 mg/kg, 2 mg/kg, or 20 mg/kg of DGAT1i or vehicle for

30 days prior to euthanasia for liver collection. Hepatic retinol and retinyl ester levels were assessed by HPLC as described above (26). Data presented as total retinol levels are the sum of retinol and retinyl esters.

Duodenal injection of oil

C57BL/6 mice ($n = 4/\text{group}$) were gavaged with either HPMC vehicle, or DGAT1i (5 mg/kg). Two hours after gavage administration, 50 μl corn oil containing retinol (6 μg) and 2×10^6 cpm [^3H]retinol was injected into the most proximal duodenum. For this procedure, mice were mildly sedated, a small abdominal incision was made, and the oil was carefully injected at the base of the stomach using a 30 gauge needle. Blood was obtained from the mice at 30 min, 1, 2, and 4 h. Mice were euthanized at 4 h after the duodenal oil injection, and plasma TG and [^3H]retinoid levels were measured as described above.

Treatment of mice with GLP-1 receptor antagonist

C57BL/6 mice were treated with DGAT1i and received an oil gavage as described above, but with both [^3H]retinol and [^{14}C]triolein tracers. To assess the effect of DGAT1i on postprandial TG metabolism independent of gastric oil retention, mice were treated with the GLP-1 receptor (GLP-1R) antagonist exendin(9-39) (Bachem, Torrance, CA) as described (33). Mice were administered exendin(9-39) (5 $\mu\text{g}/30$ g body weight) or saline (control) intraperitoneally at two time points: first, at the time of DGAT1i treatment and second, immediately following gavage with oil. Total plasma [^{14}C]triolein-derived counts and [^3H]retinoid levels were measured; gastric [^3H]retinoid and [^{14}C]triolein derived counts were analyzed as described above. Similar procedures, except with the omission of DGAT1i treatment, were also employed to assess the effect of exendin(9-39) on postprandial TG and retinoid metabolism in intestine-*Dgat1*^{-/-} mice ($n = 4/\text{group}$).

Statistics

All data are presented as mean \pm standard deviation (SD). Comparisons between two groups were performed using unpaired 2-tailed Student *t*-test. Comparisons of multiple groups were analyzed by one-way ANOVA followed by Tukey tests or two-way ANOVA followed by Bonferroni posttests.

RESULTS

Pharmacokinetic analysis of DGAT1i and its effect on postprandial TG and retinoid excursions in WT mice

To determine the optimal dose for studies of the effects of pharmacological inhibition of DGAT1, C57BL/6 mice were treated with three doses of compound 2, a pyrimidinoxazinylic bicyclooctaneacetic acid DGAT1 inhibitor (18). The plasma kinetic analysis (Fig. 1A) showed that plasma concentrations of the drug peaked at 1 h after oral administration for doses of either 5 mg/kg or 20 mg/kg, whereas levels appeared to have already declined by 1 h after administration of a dose of 1 mg/kg. The levels at each time, for each dose, are plotted relative to the IC50 of the DGAT1i in microsomal assays. On the basis of these data, we proceeded with the 5 mg and 20 mg doses, which were tested further for their effects on postprandial TG excursion. Animals were challenged with a fat load 2 h after drug treatment. The DGAT1i treatment completely abolished postprandial TG excursions over the 6 h sampling period in these animals

even though plasma concentration of the drug had already subsided by that time at the dose of 5 mg/kg (Fig. 1B). The seeming discordance between plasma levels of drug and extent of DGAT1i activity derives from blood concentrations that were greater than the IC50 throughout the 6 h period. These results are in agreement with previous reports showing that genetic ablation of *Dgat1* gene in mice results in lower plasma TG levels after a high fat challenge (11). The dose of 5 mg/kg was chosen for all other acute DGAT1i studies described in this report.

The effect of this dose on postprandial plasma excursions of TG and retinoids was dramatic; nearly complete inhibition of the corn oil-associated increases in plasma TG (Fig. 1C) and retinoids (Fig. 1D) was observed in the presence of the DGAT1i. Identical results were observed in mice that were on the WTD for 6 weeks (data not shown). The marked reductions in the postprandial plasma excursion of retinoids after DGAT1i treatment in chow-fed mice was associated with an 83% reduction in hepatic retinoid accumulation compared with vehicle-treated mice (Fig. 1E). Vehicle-treated mice had approximately 16% of the initial [^3H]retinol dose (75,716 cpm) in their livers at the end of the study, which is within normal range of hepatic uptake of dietary retinol (34). The reduction in postprandial excursion of plasma retinoids in DGAT1i-treated mice was also associated with a significant reduction (53%, $P < 0.05$) of intestinal retinoid levels (Fig. 1F). On the other hand, acute pharmacological inhibition of DGAT1 did not significantly reduce intestinal esterification of the physiologic quantity of orally administered retinol, as shown by the relative distribution of [^3H]retinol and [^3H]retinyl esters in the small intestine in vehicle-treated and DGAT1i-treated mice (Fig. 1G).

Acute DGAT1i treatment inhibits chylomicron secretion

Reduced postprandial levels of TG and retinoids could result from inhibition of chylomicron assembly and secretion or from increased fractional clearance rates, either by lipolysis or remnant clearance, of newly secreted chylomicron TG and retinyl esters. To assess whether acute inhibition of DGAT1 affects chylomicron secretion, DGAT1i-treated mice were injected with Triton WR1339 immediately before gavage of corn oil containing [^3H]triolein. In chow-fed mice, the secretion of [^3H]triolein-derived radioactivity (cpm), a marker of newly assembled chylomicrons, was inhibited by 86% ($P < 0.01$) at 4 h after the fat challenge in DGAT1i-treated mice compared with vehicle-treated mice (Fig. 2A). The apparent effect of DGAT1 inhibition on plasma TG levels was less marked because plasma TG concentrations (as opposed to radioactive TG), in the hour following Triton injection, were indicative of both intestinal and hepatic sources of TG (Fig. 2B). The latter results suggest that chylomicron secretion accounted for about one-half of the total entry of TG into the circulation during the 4 h period after Triton WR1339 injection. We assume that the rest of the increase in plasma TG over the 4 h derived from ongoing secretion of VLDL particles. However, the studies with radiolabeled, orally administered TG (Fig. 2A) indicate clearly that the DGAT1i results in marked reductions

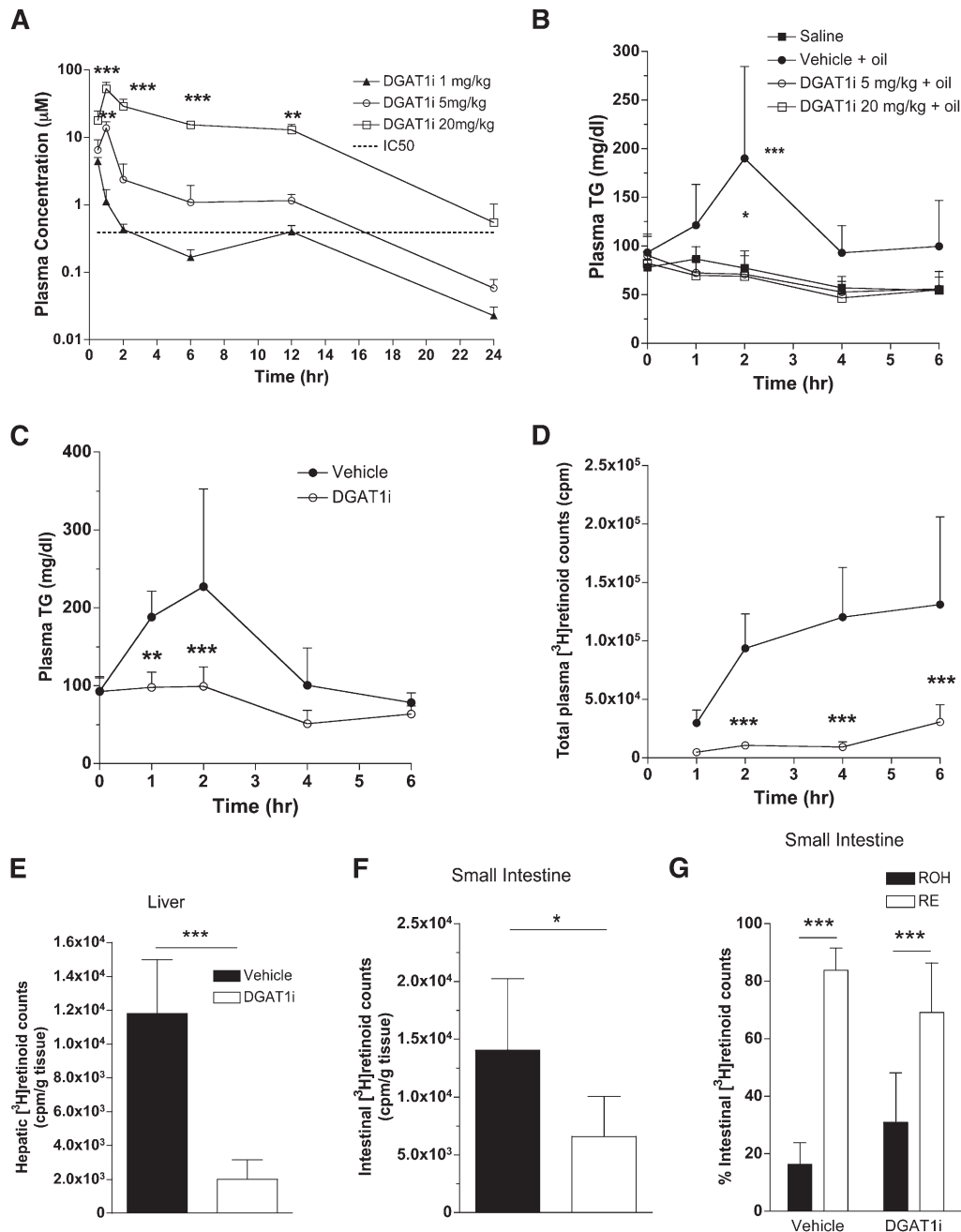


Fig. 1. Acute administration of a DGAT1i markedly reduces postprandial plasma TG and ^3H retinoid excursions and inhibits retinoid absorption by the small intestine. (A) Pharmacokinetic analysis of DGAT1i. Male C57BL/6 mice ($n = 3/\text{group}$) were gavaged with various doses of DGAT1i and plasma DGAT1i concentrations (μM) and were measured at different time points posttreatment for pharmacokinetic analysis. The *in vitro* IC₅₀ of the DGAT1i in mouse microsomal preparations (corrected for plasma protein binding) is plotted to illustrate the level of inhibitory activity to which tissues were exposed during the study. (B) Male C57BL/6 mice ($n = 6/\text{group}$) were gavaged with vehicle or DGAT1i. Two hours later, mice were gavaged with saline or corn oil. Mice were bled before (time 0) and at 1 h, 2 h, 4 h, and 6 h afterwards. Plasma samples from each time point were measured for TG levels (mg/dl). (C) Plasma TG levels (mg/dl) and (D) plasma ^3H retinoid counts (cpm) of chow-fed mice given the vehicle or 5 mg/kg DGAT1i 2 h prior to administration of 200 μl oral gavage of ^3H retinol-labeled corn oil. Mice were bled as described above and used to measure TG (mg/dl) and ^3H retinoid. Mice were euthanized at 6 h and ^3H retinoid counts (cpm/g tissue) were determined in (E) liver and (F) small intestine. (G) Retinoids were extracted from the small intestine, and the distribution of retinol and retinyl esters were determined by HPLC and expressed as percentage of total intestinal retinoid counts. $N = 6$ per group. Statistics are based on 2-way ANOVA followed by Bonferroni posttests for (A–D) and Student *t*-test for (E–G). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus vehicle-treated mice.

in the appearance of newly assembled chylomicrons from the small intestine. Because Triton WR1339 “traps” newly secreted TG-rich lipoproteins in the plasma, allowing an estimation of rates of secretion, we will hereafter describe results of the Triton WR1339 (and P-407 studies) in terms of

chylomicron secretion. This is a descriptive term and does not imply that DGAT1 plays a role within the secretory pathway. Rather, we believe that DGAT1-mediated TG synthesis is required for the assembly of chylomicrons, which is obviously essential for their secretion.

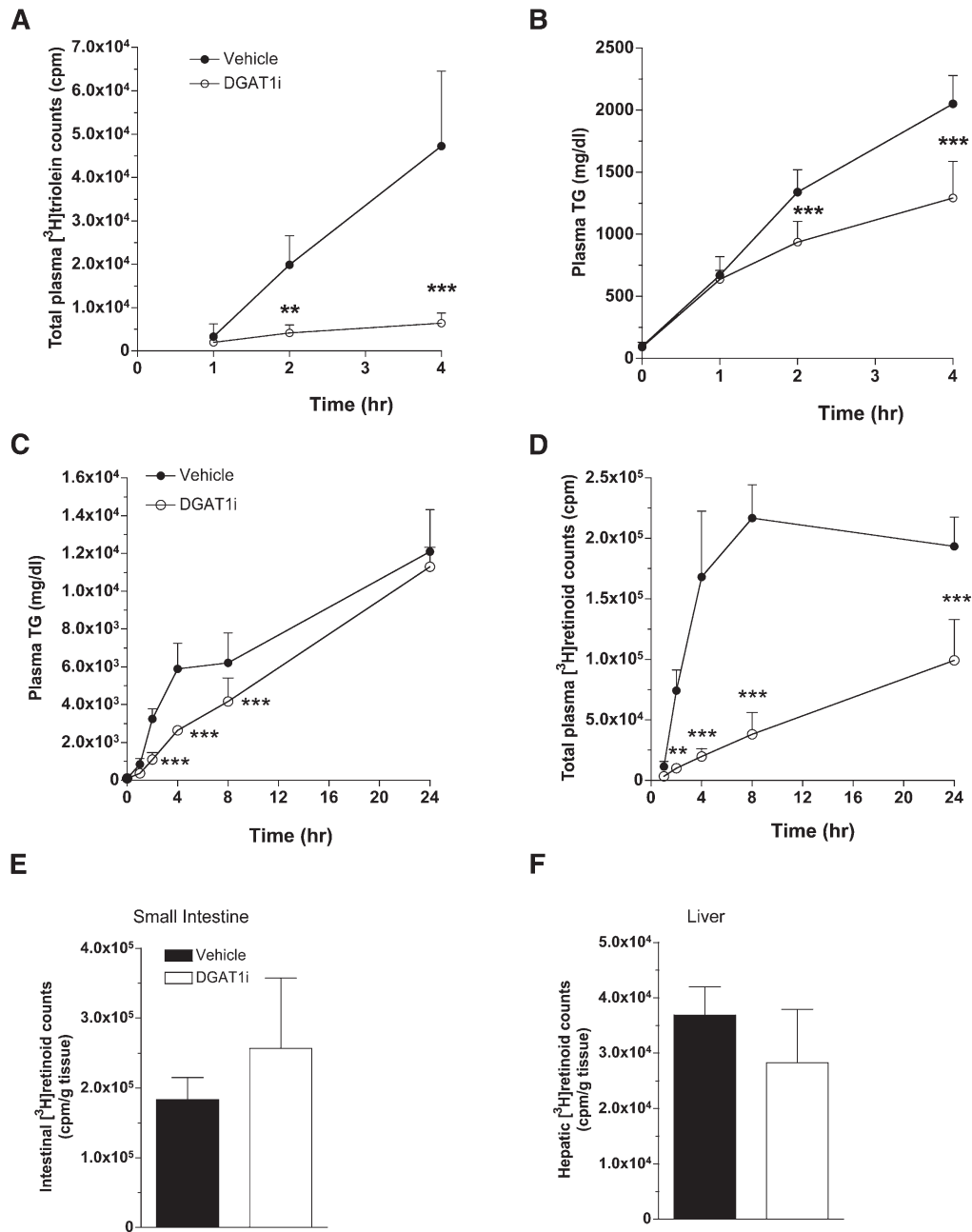


Fig. 2. Acute DGAT1i treatment inhibits chylomicron secretion in both chow-fed and WTD-fed mice. Male C57BL/6 mice ($n = 5-6$ /group) on chow diet were treated with DGAT1i or vehicle. Two hours after drug administration, mice were injected with Triton WR1339 and immediately gavaged with oil. Mice were bled at indicated time points after injection of Triton WR1339 and oil gavage. (A) Plasma ^3H triolein-derived counts were obtained at each time point. (B) Plasma TG levels (mg/dl) were measured at each time point. In a separate set of experiments, male C57BL/6 mice ($n = 5-6$ /group) on WTD diet were injected with P-407 2 h after drug or vehicle administration and immediately before oral gavage of ^3H retinol-containing oil. Mice were bled at various time points after oil gavage, and tissue was harvested at the end point of the study. (C) Plasma TG levels (mg/dl). (D) Plasma ^3H retinoid counts (cpm). (E) Total ^3H retinoid counts (cpm) per gram tissue of small intestine. (F) Total ^3H retinoid counts (cpm) per gram tissue of liver. Statistics are based on 2-way ANOVA followed by Bonferroni posttests for (A–D) and Student t -test for (E and F). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus vehicle-treated mice.

Inhibition of chylomicron secretion by acute DGAT1i is reversible by 24 h

The addition of radiolabeled retinol to the corn oil gavage allowed for a second measurement of chylomicron secretion. In addition, based on the pharmacokinetics of the DGAT1i, we wanted to determine the time course of the effects of DGAT1 inhibition on chylomicron secretion. Therefore, DGAT1i-treated mice that had been maintained on a WTD diet were administered P-407, a lipase inhibitor, prior to administration of a bolus gavage of corn oil containing [³H]retinol. As noted in *Materials and Methods*, P-407 has a long half-life, and this allowed us to follow the appearance of chylomicrons in the plasma for 24 h. Similar to chow-fed animals treated with Triton WR1339 (Fig. 2A), the accumulation of TG in the plasma after administration of P-407 was lower at both the 4 h and the 8 h time points (33% reduction, $P < 0.05$) in DGAT1i-treated mice compared with vehicle-treated animals (Fig. 2C). However, plasma TG levels between the two groups of animals were no longer significantly different 24 h after the oil gavage. These results indicate that there was no significant malabsorption of TG but, rather, a delay in the assembly and secretion of chylomicrons during the time when DGAT1i levels in blood were in the therapeutic range. However, this interpretation is confounded by increasing accumulation of hepatic-derived TG secreted in VLDL.

Inhibition of postprandial retinoid excursion paralleled that of the TG excursion up to the 8 h time point (Fig. 2D). However, plasma [³H]retinoids were still significantly lower (49% reduction, $P < 0.001$) 24 h after oil gavage, although the curves suggest that the levels would have equalized with longer observation. Importantly, intestinal and hepatic [³H]retinoid levels were no longer different at the 24 h end point (Fig. 2E, F), unlike the results after only 6 h. Together, the 4 h and the 24 h data suggest that DGAT1i treatment delayed chylomicron secretion by inhibiting intestinal [³H]retinoid (and probably TG) absorption and/or their incorporation into chylomicrons. However, this effect of the DGAT1i was reversible over a longer time period and did not, therefore, adversely affect the amount of [³H]retinoid accumulated by the liver over the course of the day. Normal levels of hepatic retinoids 24 h after administration of the DGAT1i were compatible with the results of a chronic feeding study in which WT mice were fed a WTD diet supplemented with different doses of DGAT1i for 30 days; chronic administration of the DGAT1i did not affect hepatic total retinol (retinol + retinyl ester) levels (Table 1).

DGAT1i-mediated small intestinal absorption of TG and retinol were associated with gastric retention

To determine whether DGAT1i functions similarly to the microsomal triglyceride transfer protein (MTP) inhibitor dirlotapide, which has been shown to inhibit the assembly and secretion of chylomicrons (35), mice were acutely treated with either drug or their respective vehicle controls, and then gavaged with a dose of corn oil containing [³H]retinol. Like the DGAT1i, dirlotapide abolished the postprandial TG excursion (Fig. 3A) and markedly diminished the appearance of [³H]retinoids in the plasma

(Fig. 3B). To assess the effects of dirlotapide on intestinal absorption of TG and [³H]retinol, the small intestine from each animal was analyzed in four 5 cm segments and a fifth segment representing the remainder of the small intestine. TG levels of the different intestinal segments in DGAT1i-treated mice were generally lower compared with mice given DGAT1i-vehicle, whereas the intestinal TG levels of dirlotapide-treated mice were higher compared with the other groups of animals (i.e., DGAT1i-vehicle, DGAT1i, or dirlotapide-vehicle) (Fig. 3C). Similarly, the intestinal [³H]retinoid levels in DGAT1i-treated mice were significantly lower compared with DGAT1i-vehicle-treated animals, whereas the intestinal [³H]retinoid in the dirlotapide-treated mice were significantly higher compared with the other treatment groups (Fig. 3D). Neither DGAT1i nor dirlotapide led to significant malabsorption of TG or retinoids, as no differences in colon and fecal [³H]retinoid levels were observed among the four groups of animals (Fig. 3E, F, respectively).

The differences between TG and retinoid accumulation in the small intestine after DGAT1i versus MTP inhibition were unexpected. To further elucidate the mechanisms underlying the inhibition of intestinal TG and [³H]retinol absorption after DGAT1i treatment, the gastric contents of euthanized animals were collected and the ³H-cpm levels present in these contents were measured. Fig. 3G shows that gastric [³H]retinoid levels were significantly elevated in DGAT1i-treated mice but not in dirlotapide-treated mice compared with their respective controls. Because sustained, high levels of GLP-1 can delay gastric emptying (36, 37), we measured plasma GLP-1 levels in these animals at 1 h and 6 h after gavage in DGAT1i- or dirlotapide-treated mice. Fig. 3H shows that plasma GLP-1 levels were similar in vehicle-, DGAT1i-, and dirlotapide-treated animals 1 h after delivery of corn oil by gavage. However, at 6 h postgavage, plasma GLP-1 levels in vehicle- and dirlotapide-treated mice had fallen sharply, while GLP-1 levels in DGAT1i-treated mice remained significantly elevated compared with vehicle-treated mice (167%, $P < 0.001$). Taken together, these results indicate that the decreases in intestinal and plasma levels of TG and [³H]retinoid observed after DGAT1i treatment may have resulted, at least in part, from delayed gastric emptying caused by prolonged and elevated plasma GLP-1 levels.

TABLE 1. Hepatic total retinol levels in DGAT1i-treated mice

Treatment	Dose	Total Retinol (Retinol + Retinyl Ester)
	(mg/kg)	(nmol/g liver)
Vehicle	0	305 ± 132
DGAT1i	0.2	341 ± 137
DGAT1i	2	334 ± 129
DGAT1i	20	367 ± 170

Male C57BL/6 mice (n = 6/group) were fed a WTD diet containing varying doses of an inhibitor of DGAT1 (DGAT1i) or vehicle for 30 days. Livers were assessed for retinol and retinyl ester by HPLC. Total retinol levels (nmol/g liver) are the sum of hepatic retinol and retinyl ester and expressed as mean ± SD. Comparisons were made against the vehicle group using Student *t*-test. No statistical significance was observed in comparisons made between any of the DGAT1i-WTD groups versus the vehicle group.

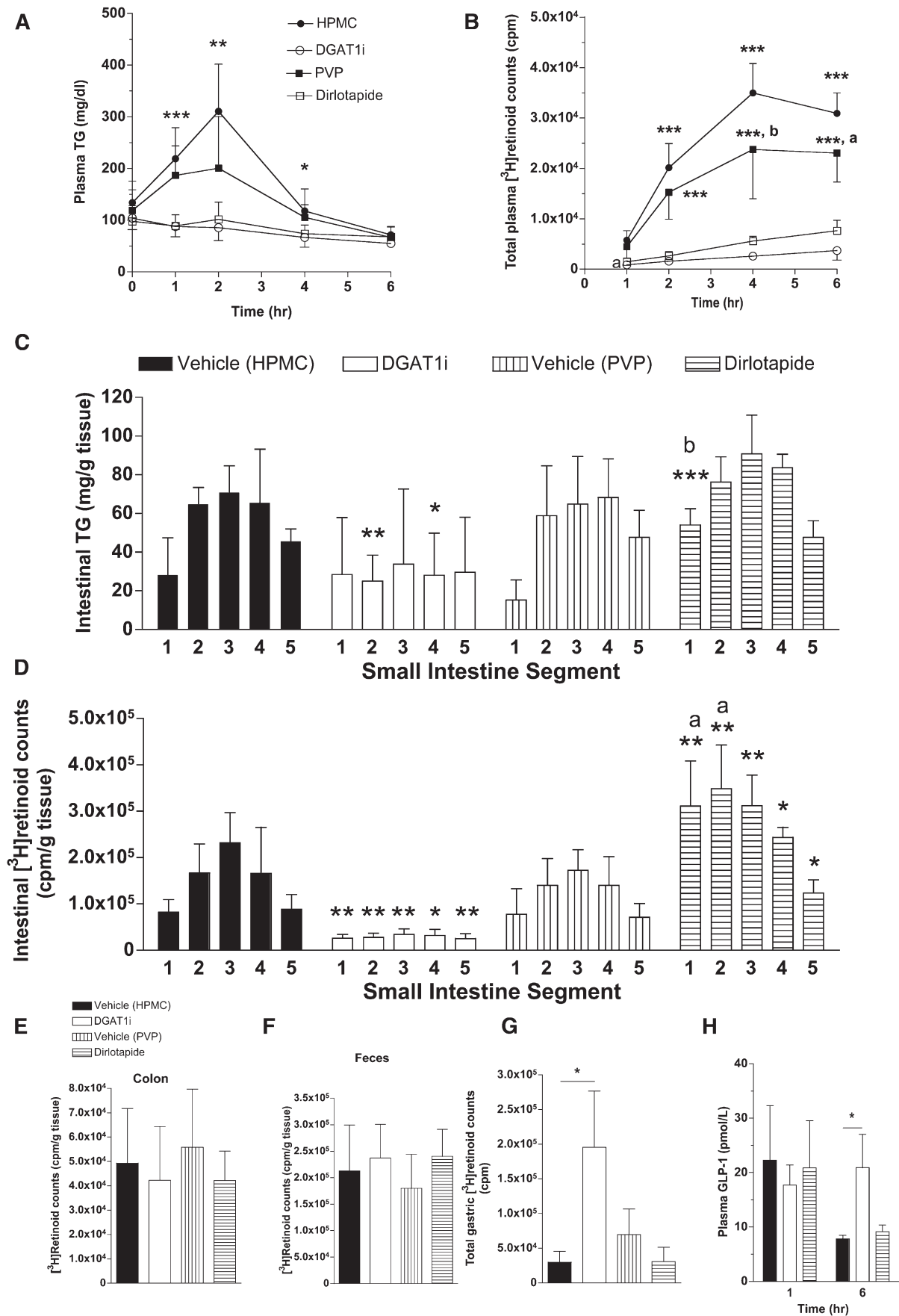


Fig. 3. Acute inhibition of DGAT1 or MTP has similar effects on plasma TG and retinoid excursions but different effects on intestinal TG and retinoid absorption. Male C57BL/6 mice ($n = 5/\text{group}$) were given HPMC, 5 mg/kg dose of DGAT1i, PVP, or 3 mg/kg dose of dirlopatide. Two hours after drug administration, mice were orally gavaged with [³H]retinol-containing corn oil. Mice were bled before (time 0)

Intestine-specific deletion of DGAT1 abolishes postprandial TG excursion and intestinal retinoid absorption and is associated with increased gastric retention

To determine whether diminished postprandial TG excursion by DGAT1i treatment results from the inhibition of DGAT1 action in the intestine alone, we performed studies in intestine-*Dgat1*^{-/-} mice and their control (*Dgat1*^{fllox/fllox}) littermates. Intestine-*Dgat1*^{-/-} mice had diminished postprandial plasma TG excursions, which were essentially identical to the responses observed in DGAT1i-treated control mice or DGAT1i-treated intestine-*Dgat1*^{-/-} mice (Fig. 4A). Similarly, the levels of postprandial plasma [³H]retinoids were diminished to the same extent in intestine-*Dgat1*^{-/-} mice and in both DGAT1i-treated control and DGAT1i-treated intestine-*Dgat1*^{-/-} mice (Fig. 4B). Furthermore, gastric retention of oil was significantly increased in intestine-*Dgat1*^{-/-} mice, and the increase was similar to DGAT1i-treated control mice (Fig. 4C). Similar to DGAT1i-treated control mice, plasma GLP-1 levels were increased in intestine-*Dgat1*^{-/-} mice (with or without DGAT1i treatment) compared with untreated control mice (Fig. 4D). Overall, these data confirm that inhibition or absence of DGAT1 only in the intestine is sufficient to abolish the postprandial TG excursion to an acute oral fat load.

Acute pharmacological inhibition of DGAT1 diminishes postprandial TG excursion and intestinal retinoid absorption independent of gastric retention

The previous experiments raised the possibility that gastric retention, via increased GLP-1, accounted for most, if not all, of the inhibition of postprandial plasma TG and retinoid excursions after an oral fat load in mice treated with DGAT1i or lacking intestinal DGAT1. To address this issue, we injected corn oil directly into the duodenum of C57BL/6 mice 2 h after administration of DGAT1i or vehicle. Postprandial plasma TG excursion was still abolished by DGAT1i treatment compared with vehicle administration, despite the fact that we had eliminated gastric retention as a potential contributor (Fig. 5A). Similarly, plasma [³H]retinoid levels were significantly lower in DGAT1i-treated mice compared with vehicle-treated mice (Fig. 5B) after duodenal administration of radiolabeled retinoid.

To further assess the significance of gastric retention in postprandial TG excursion, we inhibited endogenous signaling of the GLP-1 receptor with the antagonist exendin(9-39) (33). Blockade of GLP-1 signaling by exendin(9-39) treatment in WT C57BL/6 mice reversed the gastric retention caused by DGAT1i treatment (Fig. 6A, B). However, postprandial plasma TG (Fig. 6C), [¹⁴C]triolein-derived cpm (Fig. 6D), and [³H]retinoid (Fig. 6E) levels were reduced similarly in the groups receiving DGAT1i with or without exendin(9-39) compared with control

mice not receiving DGAT1i. Exendin(9-39) treatment did not restore intestinal absorption of [¹⁴C]triolein (Fig. 6F) or [³H]retinoid (Fig. 6G). Instead, exendin(9-39) treatment significantly increased both ¹⁴C-cpm and ³H-cpm in the colon compared with vehicle-treated control mice (Fig. 6H, I). There were no significance differences in ¹⁴C-cpm or ³H-cpm in feces (Fig. 6J, K). Together, these two alternative approaches demonstrated that inhibition of DGAT1 decreased postprandial TG and retinoid levels independent of gastric retention. However, gastric retention, by slowing the exit of fat and vitamin A from the stomach, may have reduced the quantity of triolein and retinoid that escaped the small intestine and entered the colon after an acute dose of DGAT1i.

This conclusion was further supported in a comparable study using intestine-*Dgat1*^{-/-} mice as shown in Fig. 7. Exendin(9-39) treatment reversed the gastric retention in intestine-*Dgat1*^{-/-} mice (Fig. 7A, B), but it had no effect on postprandial plasma TG (Fig. 7C), [¹⁴C]triolein-derived cpm (Fig. 7D), and [³H]retinoid (Fig. 7E), or on intestinal absorption of [¹⁴C]triolein (Fig. 7F) and [³H]retinoid (Fig. 7G). As we observed in WT mice, exendin(9-39) treatment significantly increased both ¹⁴C-cpm and ³H-cpm in the colon of intestine-*Dgat1*^{-/-} mice compared with vehicle-treated control mice (Fig. 7H, I). Unlike acute DGAT1i inhibition, chronic DGAT1 deficiency not only increased the passage of dietary fat into colon but also fecal excretion of dietary fats (Fig. 7J, K), which was not affected by exendin(9-39) treatment.

Genetic ablation of DGAT1 causes chronic inhibition of chylomicron secretion in intestine-*Dgat1*^{-/-} mice, and this inhibition is not corrected by restoring gastric retention

Our earlier studies showed that acute DGAT1i treatment inhibited chylomicron secretion in C57BL/6 WT mice on WTD (Fig. 1) and that this inhibition was reversible by 24 h when DGAT1i was no longer effective in these animals (Fig. 2). Those observations are complemented by studies in chow-fed intestine-*Dgat1*^{-/-} mice (Fig. 8). Upon P-407 injection, chylomicron secretion was significantly reduced in chow-fed intestine-*Dgat1*^{-/-} mice throughout the entire 24 h time course. Plasma TG (Fig. 8A) and plasma [¹⁴C]triolein (Fig. 8B) levels remained significantly reduced compared with levels in control littermates. Similar to acute DGAT1i administration in WT mice, plasma [³H]retinoid counts were significantly lower in intestine-*Dgat1*^{-/-} mice compared with those in control littermates (Fig. 8C). The results in Fig. 8B, depicting [¹⁴C]triolein-derived radioactivity, also better clarify the long-term effects of intestinal DGAT1 deficiency compared with Fig. 2B. Additionally, reversal of gastric retention defect in intestine-*Dgat1*^{-/-} mice by exendin(9-39) treatment did not have any effect on chylomicron secretion. It is noteworthy, however, that

and at various time points for 6 h after fat challenge. Plasma TG levels (mg/dl) (A) and plasma [³H]retinoid counts (cpm) (B) were measured. At the end point, five segments of intestines were used to measure intestinal TG (mg/g tissue) (C) and intestinal [³H]retinoid counts (cpm/g tissue) (D). [³H]Retinoid counts (cpm/g tissue) taken from whole colon (E), feces (F), and gastric contents (G) were also measured. (H) Plasma GLP-1 levels (pmol/l) of mice bled at 1 and 6 h after oil gavage. Statistics are based on 2-way ANOVA followed by Bonferroni posttests for (A and B) and Student *t*-test for (C–H). **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus vehicle-treated mice; ^a*P* < 0.05 versus DGAT1i; and ^b*P* < 0.01 versus HPMC.

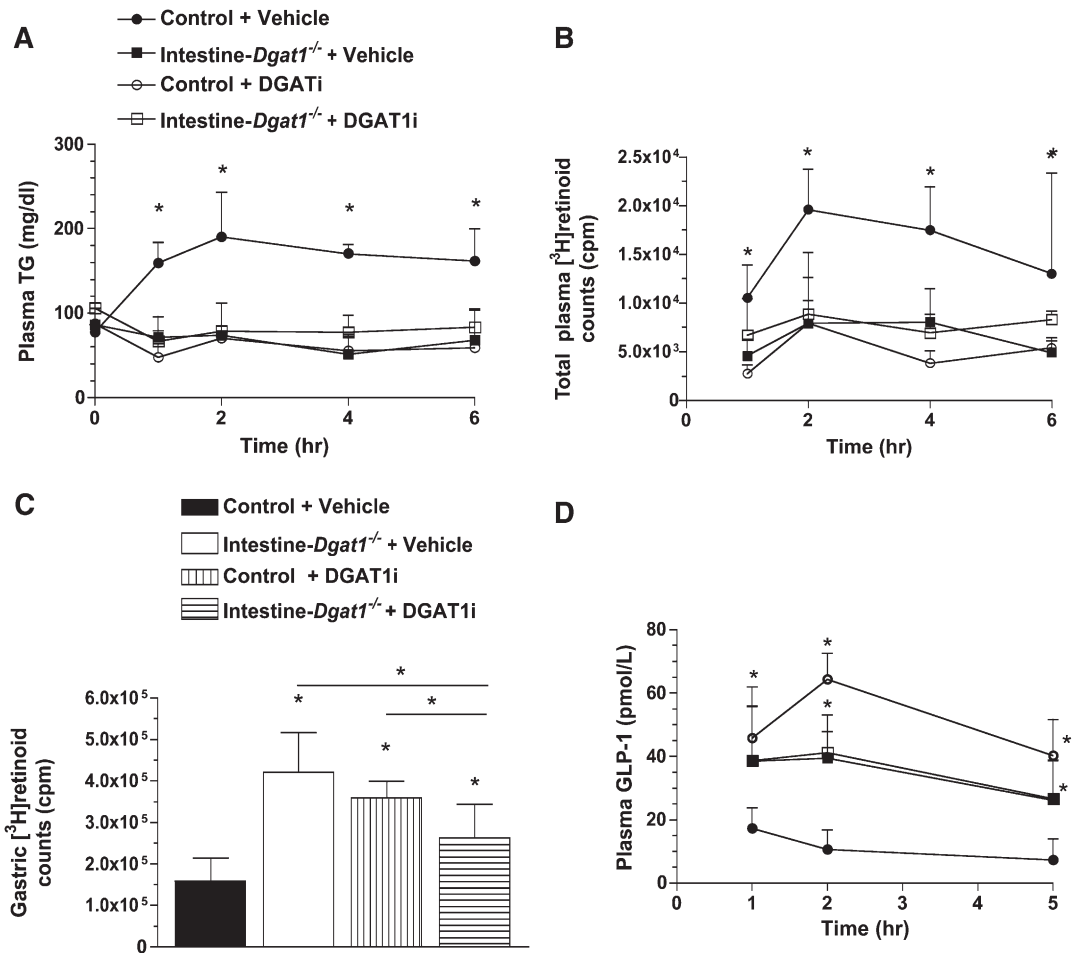


Fig. 4. Mice with intestine-specific deletion of DGAT1 expression have decreased postprandial TG and [³H]retinoid levels as well as delayed gastric emptying after a fat load. Male intestine-*Dgat1*^{-/-} mice (n = 4/group) and their control littermates (n = 4/group) were treated with vehicle or DGAT1i. All mice were gavaged with [³H]retinol-containing corn oil 2 h after drug administration. Mice were bled before or after oil gavage at the indicated time points. (A) Plasma TG (mg/dl) and (B) plasma [³H]retinoid counts (cpm) were measured over 6 h. (C) [³H]retinoid counts (cpm) of gastric contents were obtained after mice were euthanized at 6 h. (D) Plasma GLP-1 (pmol/L) was measured over the course of the studies. Statistics are based on 1-way ANOVA followed by Tukey posttests. **P* < 0.05 versus vehicle-treated control mice or, when indicated, between two experimental groups (C).

there was a steady, albeit reduced, appearance of both [¹⁴C]triolein-derived and [³H]retinoid radioactivity in plasma over the 24 h sampling period.

DISCUSSION

Demonstration that genetic ablation of DGAT1 in mice protects against high-fat diet-induced obesity and insulin resistance (11, 14, 15) provided incentives for development of specific DGAT1 inhibitors, some of which have reproduced aspects of the *Dgat1*^{-/-} phenotype in animal studies (17). In this report, we have extensively investigated the effects of a small-molecule DGAT1i on intestinal TG and retinoid metabolism in both WT mice and mice lacking DGAT1 specifically in the intestine. We chose to focus on the intestine because *i)* DGAT1 is the major enzyme that catalyzes the final step in TG synthesis in the small intestine; *ii)* we were concerned that DGAT1i, by inhibiting chylomicron assembly and secretion, could impact negatively on the delivery of dietary retinol to the rest

of the body; and *iii)* a recent report by Lee et al. (38) indicated that much of the overall phenotype in the total body *Dgat1*^{-/-} mouse could be reversed by expressing *Dgat1* in the small intestine.

In agreement with previous studies (18–21), including a very recent report (22), we showed that the acute administration of a small-molecule DGAT1i effectively blocked postprandial plasma TG excursions after an oral fat challenge in both chow- and WTD-fed mice. This effect of DGAT1i resulted from nearly complete blockade of chylomicron secretion, as defined by the appearance of both unlabeled and labeled TG after gavaging mice with [³H]triolein-containing oil and inhibiting lipolysis with Triton WR1339. Our results are consistent with those of Buhman et al. (16), although those authors did not conduct experiments in which lipolysis was inhibited and could not, therefore, rule out an effect of DGAT1i on clearance of chylomicrons TG from the circulation. The effects of DGAT1i on postprandial lipemia are consistent with the importance of TG synthesis for the assembly and secretion of chylomicrons, as demonstrated in studies of *Dgat1*^{-/-} mice

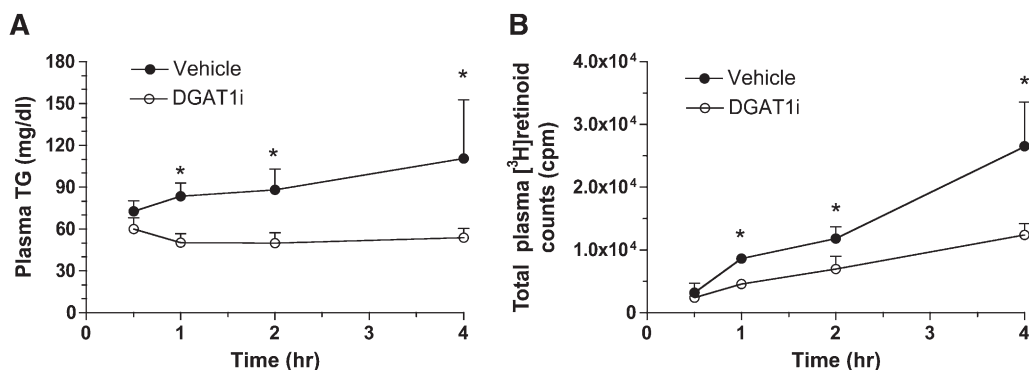


Fig. 5. Duodenal injection of oil in DGAT1i-treated mice show that delayed gastric emptying does not explain the reductions in postprandial plasma TG and retinoid excursions. Male C57BL/6 mice ($n = 4/\text{group}$) were treated with DGAT1i or vehicle and oil injected directly into the duodenum 2 h after drug administration. Mice were bled before or after oil gavage at the indicated time points. (A) Plasma TG (mg/dl). (B) Total plasma [³H]retinoid counts (cpm). Statistics are based on 1-way ANOVA followed by Tukey posttests. * $P < 0.05$ versus vehicle-treated mice.

(11, 39), *Mgat2*^{-/-} mice (39), MTP inhibitors (40), and patients with abetalipoproteinemia (41).

DGAT1i treatment also dramatically diminished postprandial plasma retinoid excursion, a finding also reported by Buhman et al. (16). While this might have been expected, considering that retinyl esters are carried from the intestine to the liver on chylomicrons, it is also possible that the reduction in plasma retinoid levels with DGAT1i treatment resulted from inhibition of the ARAT activity associated with DGAT1 (26). However, the relative distribution of [³H]retinol and [³H]retinyl esters in the small intestine of DGAT1i-treated mice was similar to that observed in control mice, in agreement with earlier published studies demonstrating that the majority of intestinal retinoid esterification under physiological conditions is catalyzed by LRAT (23, 26).

This conclusion is further supported by the normal distribution of [³H]retinol and [³H]retinyl esters in plasma after 6 h, when total retinoid levels were still markedly reduced (data not shown), and the gradual increases of [³H]retinoids in the plasma as the DGAT1i lost effectiveness in association with its catabolism. Indeed, at 24 h after DGAT1i treatment, liver [³H]retinoid levels were similar in control and inhibited mice. It would appear, therefore, that DGAT1i delays or slows, but does not significantly diminish, total transport of oral retinoid from the intestine to the liver. Some malabsorption was noted, however, in WT mice treated with DGAT1i and exendin(9-39) and in the intestine-*Dgat1*^{-/-} mice.

That the majority of retinoid is finally absorbed and secreted into the plasma is also consistent with the data in Table 1, which show that chronic administration of the DGAT1i does not result in significantly diminished hepatic retinoid stores even after 30 days of continuous administration of the drug. Furthermore, hepatic total retinoid levels are identical in age-, gender-, diet-, and genetic background-matched WT and *Dgat1*^{-/-} mice (26). Buhman et al. (16) suggested that formation of diacylglycerol via MGAT, followed by transacylation of fatty acids could provide TG for chylomicron formation; this pathway might be slower but could account for delayed but significant retinoid absorption when DGAT1 is inhibited. It is also possible that there

is another pathway for retinoid absorption that is not via chylomicrons; this alternative pathway could also be slower than the chylomicron route of transport. Such a pathway might be via HDL or might entail retinol or retinyl ester direct absorption into the portal system. The success of replacement therapy with oral retinoids in patients with abetalipoproteinemia supports the existence of such a secondary pathway(s) for acquisition of essential fat-soluble vitamins.

Our finding of reduced levels of retinoids in the small intestine 6 h after corn oil gavage with normal distribution of retinol and retinyl esters was unexpected. If LRAT activity was able to normally esterify retinol but chylomicron secretion was markedly reduced because of the absence of newly synthesized TG, retinyl esters should have accumulated in the small intestine. Therefore, we conducted a comparative study in mice treated with either an inhibitor of MTP or DGAT1. Both DGAT1 and MTP inhibitors similarly inhibited postprandial TG excursion and the appearance of [³H]retinoids in the plasma, but they exerted distinct effects on the absorption and accumulation of TG and retinoids in the small intestine. The MTP inhibitor not only abolished the secretion of chylomicrons but, as expected from studies of the small intestine in mice lacking MTP (42) or individuals with abetalipoproteinemia (41), caused the accumulation of TG and [³H]retinoids in the proximal 5–10 cm of the small intestine. In contrast, there was no increase in TG or [³H]retinoids across the entire small intestine in DGAT1i-treated animals after an oral fat challenge; in fact, their levels were reduced. In a recent report characterizing another DGAT1i, histology of the jejunum 8 h after gavage with an undefined quantity of corn oil demonstrated more neutral lipid staining with the inhibitor-treated mice (22); our studies demonstrating decreased intestinal TG and retinoid levels (Figs. 3, 6, and 7) were at 6 h with 200 μl corn oil. GLP-1 has been shown to inhibit gastric emptying in humans (36, 37), and a previous study has shown that there was an exaggerated response of GLP-1 levels and a concomitant delay in gastric emptying in *Dgat1*^{-/-} mice (39). In this report, we found that DGAT1i treatment caused a prolonged increase

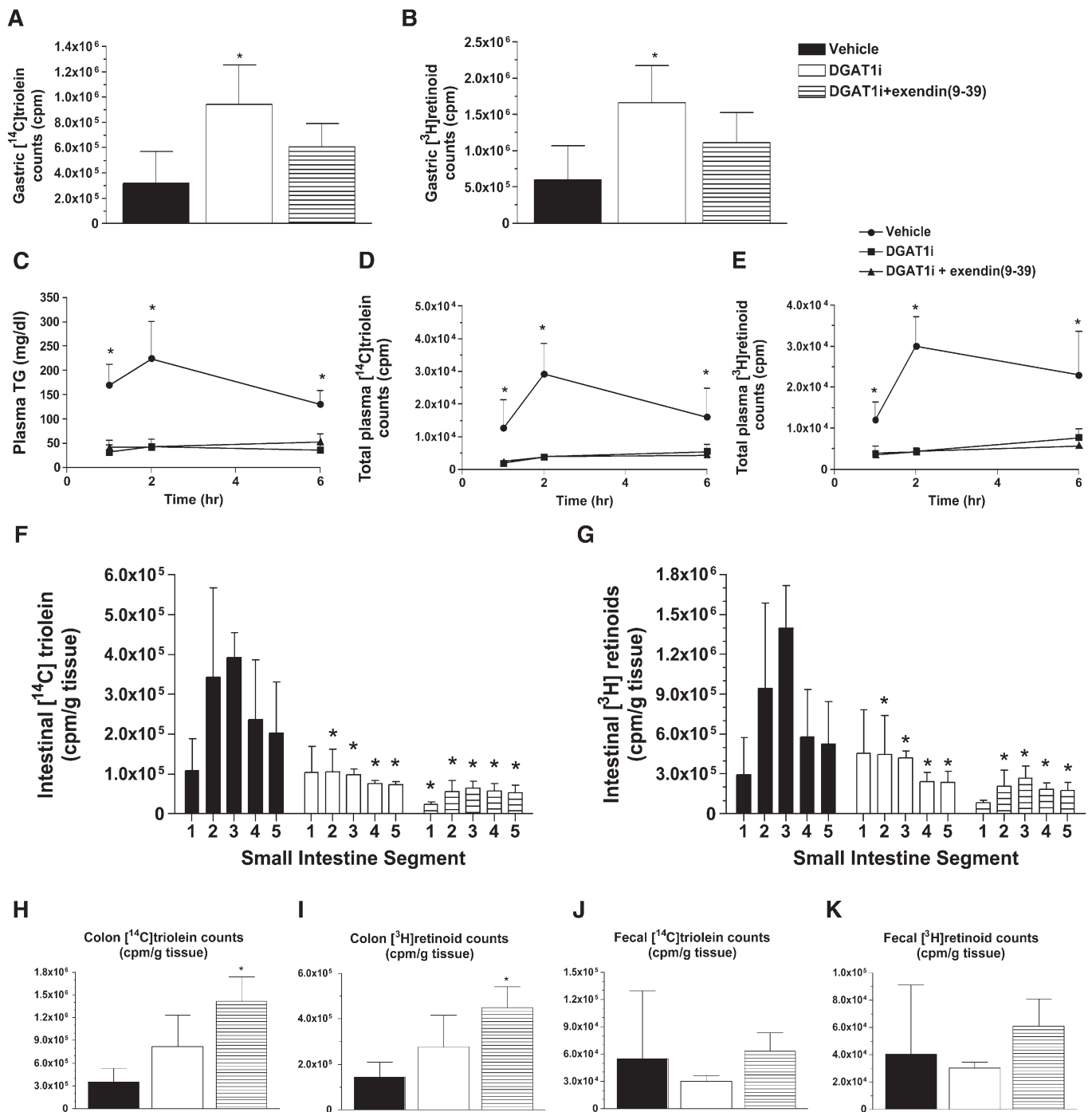


Fig. 6. Coadministration of DGAT1i with a GLP-1R antagonist demonstrates that delayed gastric emptying does not explain the reductions in postprandial plasma TG and retinoid excursions in C57BL/6 mice. Male C57BL/6 mice ($n = 4/\text{group}$) were treated with DGAT1i or HPMC vehicle. Exendin(9-39) was administered intraperitoneally at the time of DGAT1i treatment and at the time of oil gavage. The oil contained both ^{14}C triolein and ^3H retinol tracers. Mice were bled before or after oil gavage at the indicated time points. Mice were euthanized at 6 h, and gastric contents were collected for measurement of ^{14}C triolein-derived counts (A) and ^3H retinoid counts (B). Plasma samples from various time points were measured for plasma TG (C), total plasma ^{14}C triolein-derived counts (D), and total plasma ^3H retinoid counts (E). Five segments of intestines were used to measure intestinal ^{14}C triolein-derived counts (F) and intestinal ^3H retinoid counts (G). Whole colon was measured for ^{14}C triolein-derived counts (H) and ^3H retinoid counts (I). Feces were also measured for ^{14}C triolein-derived counts (J) and ^3H retinoid counts (K). Statistics are based on 1-way ANOVA followed by Tukey posttests. * $P < 0.05$ versus HPMC vehicle-treated mice.

in plasma GLP-1 levels after a fat challenge to WT mice. Additionally, both untreated and DGAT1i-treated intestine-*Dgat1*^{-/-} mice showed significant gastric retention 6 h after a fat challenge compared with untreated WT mice.

Overall, these data suggest that DGAT1 may act directly to regulate GLP-1 release from intestinal L-cells after fat ingestion. Although the mechanism underlying this regulation is undefined, two possibilities can be considered. First,

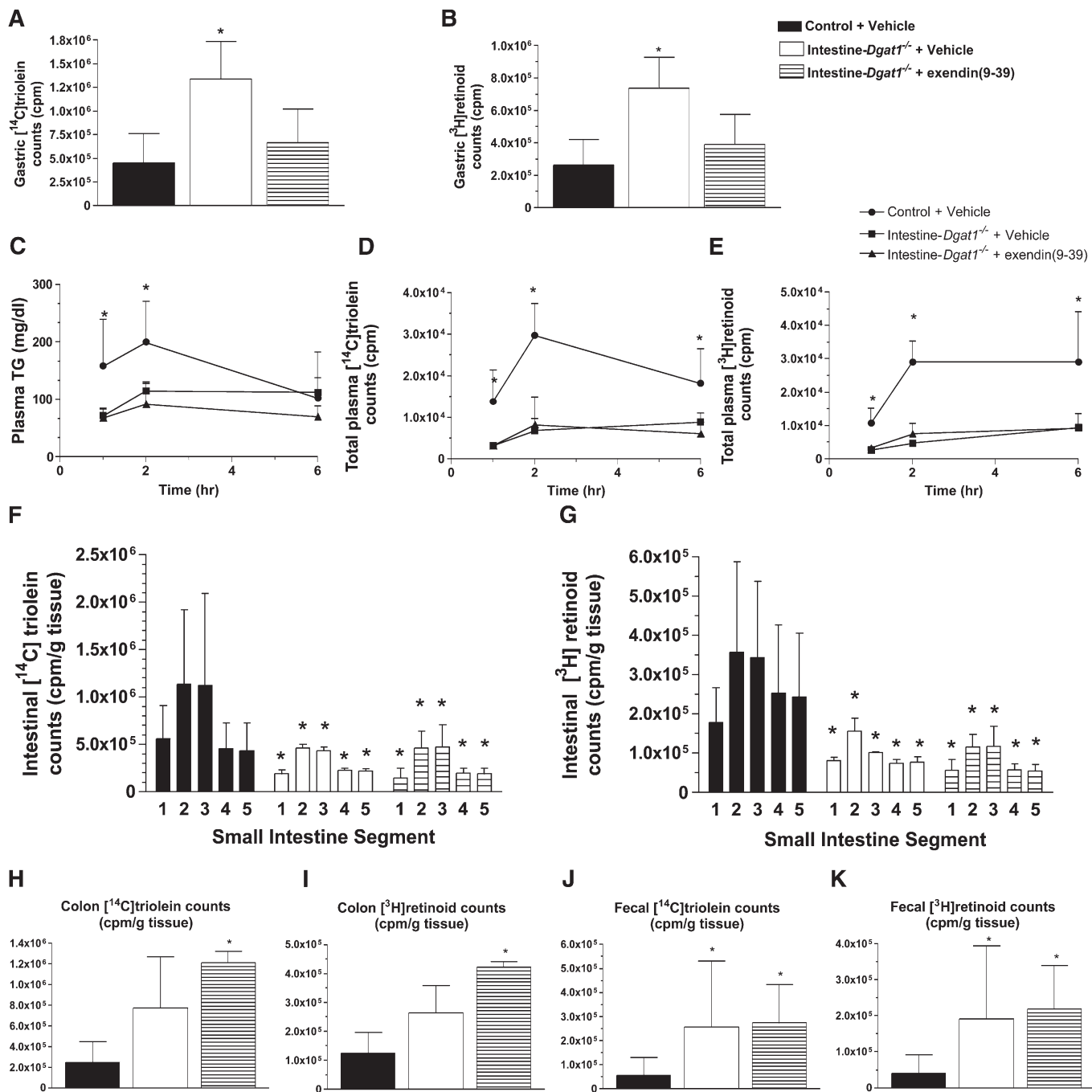


Fig. 7. GLP-1R antagonist treatment corrects gastric retention, but not postprandial plasma TG and retinoid excursions in intestine-*Dgat1*^{-/-} mice. Male C57BL/6 mice were treated with vehicle and intestine-*Dgat1*^{-/-} mice ($n = 4/\text{group}$) were treated with either DGAT1i or vehicle. Exendin(9-39) was administered intraperitoneally at the time of DGAT1i treatment and at the time of oil gavage. The oil contained both [¹⁴C]triolein and [³H]retinoid tracers. Mice were bled before or after oil gavage at the indicated time points. Mice were euthanized at 6 h, and gastric contents were collected for measurement of [¹⁴C]triolein-derived counts (A) and [³H]retinoid counts (B). Plasma samples from various time points are measured for plasma TG (C), total plasma [¹⁴C]triolein-derived counts (D), and total plasma [³H]retinoid counts (E). Five segments of intestines were used to measure intestinal [¹⁴C]triolein-derived counts (F) and intestinal [³H]retinoid counts (G). Whole colon was measured for [¹⁴C]triolein-derived counts (H) and [³H]retinoid counts (I). Feces were also measured for [¹⁴C]triolein-derived counts (J) and [³H]retinoid counts (K). Statistics are based on 1-way ANOVA followed by Tukey posttests. * $P < 0.05$ versus control mice.

DGAT1 activity has been identified in L-cells, the site of intestinal GLP-1 production; inhibition of DGAT activity in L-cells could alter GLP-1 secretion (39). Second, inhibition of DGAT1 in the upper regions of the small intestine could result in increased absorption of fatty acids and

monoglycerides in the low regions where L-cells reside; this could increase GLP-1 release independent of any effects on L-cell DGAT1 activity. Irrespective of the mechanism, the ability of DGAT1 inhibitors to increase GLP-1 secretion suggests that this class of agents may have effects

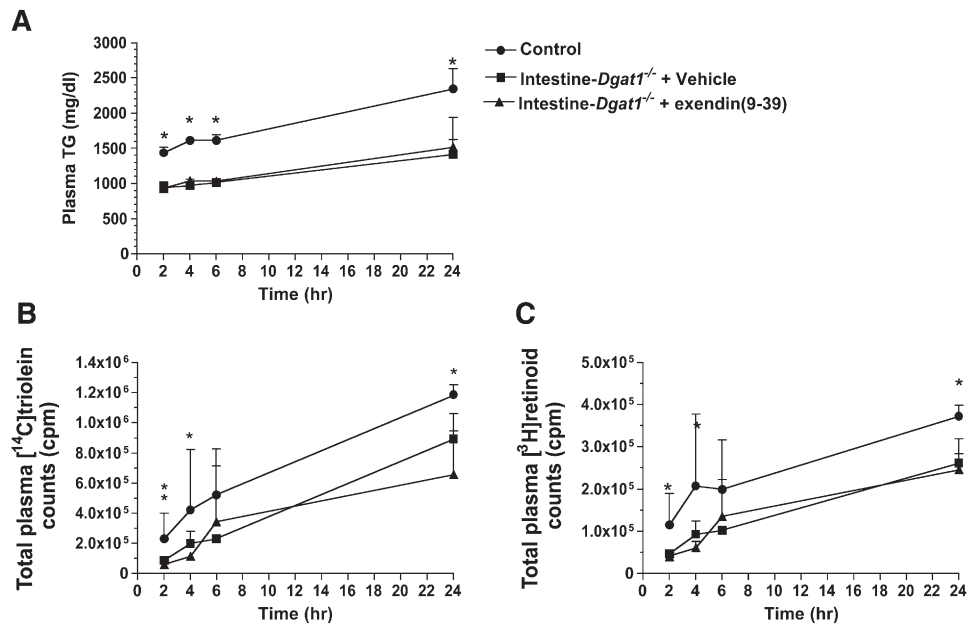


Fig. 8. Chylomicron secretion in intestine-*Dgat1*^{-/-} mice is chronically inhibited. Chow-fed male intestine-*Dgat1*^{-/-} and their control littermates (n = 4/group) were administered with P-407 intraperitoneally 5 min prior to an oil gavage. Intestine-specific *Dgat1*^{-/-} mice also received exendin(9-39) or vehicle intraperitoneally at the time of gavage. Mice were bled at various time points after oil gavage. (A) Plasma TG levels (mg/dl). (B) Total plasma [¹⁴C]triolein-derived counts (cpm). (C) Total plasma [³H]retinoid counts (cpm). Statistics are based on 1-way ANOVA followed by Tukey posttests. **P* < 0.05, ***P* < 0.01 versus control mice.

on satiety, weight loss, and insulin secretion similar to those demonstrated for GLP-1 agonists.

The observation of increased GLP-1 levels raised a critical question: Could the marked reductions in postprandial plasma levels of TG and retinoids be explained completely by GLP-1-induced gastric retention, or did inhibition of DGAT1 have a direct effect on chylomicron assembly and secretion? Our demonstration of significant inhibition of postprandial TG and retinoid excursions in mice injected with oil directly in duodenum, thus bypassing the stomach, and the potential effect of DGAT1i-induced gastric retention, point clearly to direct effects of DGAT1 inhibition. This conclusion is supported by our second approach, in which we inhibited the interaction of GLP-1 with the GLP-1R by coadministering exendin(9-39), a competitive antagonist to GLP-1 (33); we observed marked reductions in plasma appearance of TG and [³H]retinoids in DGAT1i-treated mice despite near normalization of gastric emptying. Importantly, recent studies have indicated that pharmacological activation of GLP-1R inhibits chylomicron and VLDL secretion in mice (33, 43). The fact that we observed inhibition of chylomicron secretion when we blocked GLP-1 action with exendin(9-39) is further evidence supporting a direct effect of DGAT1 inhibition to reduce chylomicron secretion. Altogether, these experiments indicate that although DGAT1i-induced gastric retention can play a role in the acute inhibition of chylomicron secretion, it is not necessary for the efficacy of the DGAT1i. Our studies in the intestine-*Dgat1*^{-/-} with and without exendin(9-39) provide clear, further evidence for the essential role of DGAT1 in chylomicron formation and secretion.

In summary, we have demonstrated that pharmacological inhibition of postprandial plasma TG and retinoid excursions results from the direct inhibitory effect of the drug on intestinal DGAT1-mediated TG synthesis, resulting in impaired chylomicron assembly and secretion. The reduction in postprandial retinoid levels was likely due to decreased chylomicron assembly. We also show that acute DGAT1i-treatment results in a delay in gastric emptying upon a fat challenge; this increases the acute efficacy of the drug but is not necessary for the majority of its activity. Furthermore, we found that the alterations induced by DGAT1i in intestinal lipid metabolism and gastric motility were the same as those present in intestine-*Dgat1*^{-/-} mice. Finally, although the long-term effects of pharmacological use of DGAT1 inhibitors on vitamin A absorption in humans are not yet known, our data obtained in mice establish that chronic inhibition of DGAT1 does not directly inhibit either intestinal retinyl ester synthesis or hepatic retinoid accumulation. **FIG**

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