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DGAT1 Is Not Essential for Intestinal Triacylglycerol Absorption or Chylomicron Synthesis*

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Dietary triacylglycerols are a major source of energy for animals. The absorption of dietary triacylglycerols involves their hydrolysis to free fatty acids and monoacylglycerols in the intestinal lumen, the uptake of these products into enterocytes, the resynthesis of triacylgylcerols, and the incorporation of newly synthesized triacylglycerols into nascent chylomicrons for secretion. In enterocytes, the final step in triacylglycerol synthesis is believed to be catalyzed primarily through the actions of acyl-CoA:diacylglycerol acyltransferase (DGAT) enzymes. In this study, we analyzed intestinal triacylglycerol absorption and chylomicron synthesis and secretion in DGAT1-deficient (*Dgat1*$^{-/-}$) mice. Surprisingly, DGAT1 was not essential for quantitative dietary triacylglycerol absorption, even in mice fed a high fat diet, or for the synthesis of chylomicrons. However, *Dgat1*$^{-/-}$ mice had reduced postabsorptive chylomicronemia (1 h after a high fat challenge) and accumulated neutral-lipid droplets in the cytoplasm of enterocytes when chronically fed a high fat diet. These results suggest a reduced rate of triacylglycerol absorption in *Dgat1*$^{-/-}$ mice. Analysis of intestine from *Dgat1*$^{-/-}$ mice revealed activity for two other enzymes, DGAT2 and diacylglycerol transacylase, that catalyze triacylglycerol synthesis and apparently help to compensate for the absence of DGAT1. Our findings indicate that multiple mechanisms for triacylglycerol synthesis in the intestine facilitate triacylglycerol absorption.

The absorption of triacylglycerols by the intestine is highly efficient, and more than 95% of dietary triacylglycerols is absorbed, even if the diet is rich in fat. By comparison, only 30–70% of dietary cholesterol is absorbed in most animals (1). The high efficiency of triacylglycerol absorption is likely due to an evolutionary pressure that maximized the ability to absorb rich sources of energy (such as fat) when food sources were scarce.

Intestinal triacylglycerol absorption occurs by a series of steps in which dietary triacylglycerols are first hydrolyzed in the intestinal lumen and then resynthesized within enterocytes. In the lumen, dietary triacylglycerols are hydrolyzed by lipases to generate free fatty acids and monoacylglycerols. These molecules are taken up by enterocytes and then enter the triacylglycerol biosynthesis pathways. The triacylglycerol products are incorporated into nascent chylomicrons, which are subsequently secreted from enterocytes and enter the lymphatic system.

Triacylglycerol biosynthesis in the intestine is believed to occur mainly through the monoacylglycerol pathway. In this pathway, monoacylglycerol and fatty acyl-CoA are covalently joined to form diacylglycerol in a reaction catalyzed by monoacylglycerol acyltransferase (MGAT)[1] (2). Diacylglycerol and fatty acyl-CoA are then used to synthesize triacylglycerol in a reaction catalyzed by acyl-CoA:diacylglycerol acyltransferase (DGAT) enzymes. High levels of DGAT activity are present in the small intestine (3–5), and both known DGAT genes, *Dgat1* and *Dgat2*, are expressed in this tissue (6, 7). In addition, a fatty acyl moiety from one diacylglycerol can be transferred to a second diacylglycerol to synthesize triacylglycerol in a reaction catalyzed by diacylglycerol transacylase. This activity has been reported in rat small intestine (8). The relative contributions of DGAT1, DGAT2, and diacylglycerol transacylase in intestinal triacylglycerol synthesis and absorption are unknown.

To investigate the biological functions of DGAT1, we generated DGAT1-deficient (*Dgat1*$^{-/-}$) mice (9). These mice are viable and healthy, in part because they can synthesize triacylglycerols through alternative mechanisms. However, they have reduced body triacylglycerol content and are resistant to obesity through a mechanism involving increased energy expenditure (9). Surprisingly, despite a marked reduction in DGAT activity in *Dgat1*$^{-/-}$ intestine (9), we found no evidence of overt fat malabsorption in the initial characterization of *Dgat1*$^{-/-}$ mice.

In this study, we used *Dgat1*$^{-/-}$ mice to further define the function of DGAT1 in the intestine. We sought to determine

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[1] The abbreviations used are: MGAT, monoacylglycerol acyltransferase; DGAT, acyl-CoA:diacylglycerol acyltransferase; BMIPP, 15-(*p*-iodophenyl)-3-(*R,S*)-methylpentadecanoic acid.

whether DGAT1 deficiency alters triacylglycerol metabolism in enterocytes and whether *Dgat1*$^{-/-}$ mice can synthesize chylomicron-sized lipoprotein particles. We also investigated the relative contributions of DGAT1, DGAT2, and diacylglycerol transacylase in intestinal triacylglycerol synthesis and absorption.

EXPERIMENTAL PROCEDURES

Mice and Diets—Dgat1$^{-/-}$ mice in a C57BL/6J background were generated and genotyped as described (9). Wild-type (*Dgat1*$^{+/+}$) mice in a C57BL/6J background were from the Jackson Laboratory (Bar Harbor, ME). All mice were housed in a pathogen-free barrier facility (12-h light/12-h dark cycle) and fed either rodent chow containing 4.5% fat (Picolab 20, Ralston-Purina, St. Louis, MO), a synthetic, high milk fat diet containing 20% (w:w) anhydrous milk fat and 0.15% cholesterol (Harlan Teklad, Madison, WI) or a synthetic, high corn oil diet containing 20% (w:w) corn oil and 0.15% cholesterol (Harlan Teklad).

Dietary Fat Absorption—Dietary fat absorption was measured by a modification of a fecal isotope ratio method used to measure cholesterol absorption (10, 11). In brief, [^{14}C]oleic acid (25 μCi) (Amersham Biosciences) and [5,6-^{3}H]sitostanol (10 μCi) (American Radiolabeled Chemicals, St. Louis, MO), a nonabsorbed standard, in 15 ml of safflower oil were administered intragastically to nonfasted mice by gavage. Feces were then collected for 5 days, and the ratio of ^{14}C to ^{3}H radioactivity in aliquots of fecal extracts was used to calculate the percent dietary fat absorption.

Intestinal Gene Expression—Intestines from nonfasted wild-type mice were divided into five sections of equal length (section 1 most proximal and section 5 most distal to the stomach). Total RNA was extracted from intestinal mucosa with RNA STAT60 (Tel-Test, Friendswood, TX), and equivalent amounts of RNA were pooled from four mice for mRNA purification (oligo(dT)-cellulose columns, Amersham Biosciences). Northern analysis was performed with 5 μg of mRNA and ^{32}P-labeled cDNA probes for *Dgat1* and *Dgat2*. Membranes were reprobed for actin to normalize for sample loading differences, and signals were quantified with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

In Situ Hybridization—In situ hybridization was performed as described (12). Briefly, intestine sections from wild-type mice were deparaffinized and fixed in 4% paraformaldehyde. After proteinase K digestion, the sections were hybridized at 55 °C for 12 h with ^{35}S-labeled antisense or sense *Dgat1* RNA probes. The probe spans nucleotides 371–1089 of the *Dgat1* coding sequence in GenBank™ (accession number AF078752). The sections were washed for 20 min in 2× SSC, 10 mM β-mercaptoethanol, and 1 mM EDTA, treated with RNase A (20 μg/ml), and washed at high stringency (0.1× SSC, 10 mM β-mercaptoethanol, and 1 mM EDTA) for 2 h at 60 °C. The sections were dehydrated, dipped in photographic emulsion NTB_2 (Eastman Kodak), and stored at 4 °C. After 8 weeks of exposure, the sections were developed and counterstained with hematoxylin and eosin.

Postabsorptive Plasma Analyses—A bolus of lipid (100 μl of corn oil) was administered intragastrically to fasted (4 h) mice by gavage. Blood samples were obtained from the retro-orbital plexus before and 1 h after lipid administration. Plasma triacylglycerols were measured with a colorimetric assay (Triglycerides/GB kit, Roche Molecular Biochemicals). Chylomicrons and very low density lipoproteins ($d < 1.006$ g/ml) were isolated from pooled plasma samples ($n = 4$ mice) by ultracentrifugation (50,000 × g at 4 °C for 30 min). In other experiments, plasma lipoproteins were separated by agarose gel electrophoresis (13) and stained for lipid with Fat Red 7B (Sigma). Chylomicron staining was quantified by densitometry.

Absorption of Vitamins—Retinol palmitate absorption was determined as described (14). Briefly, 5000 units of retinol palmitate (Sigma) mixed in 100 μl of corn oil was administered intragastically to fasted mice (4 h) by gavage. Blood samples were obtained before and 2 h after administration for analysis. Plasma retinol levels were analyzed with reversed-phase liquid chromatography. Plasma α-tocopherol levels were measured in nonfasted mice with a modified method of Lang *et al.* (15).

Absorption of the Nonoxidizable Fatty Acid BMIPP—[^{125}I]15-(*p*-Iodophenyl)-3-(*R,S*)-methylpentadecanoic acid (BMIPP) was prepared by the thallation-iodide exchange method as described (16). BMIPP was purified with a Sep-Pak RP-18 Light cartridge (Waters, Milford, MA). The specific activity of [^{125}I]BMIPP was typically 2–4 Ci/mmol. BMIPP absorption was determined by measuring the appearance of radioactivity into plasma triacylglycerols in mice after an overnight fast (15 h). [^{125}I]BMIPP (20 μCi) was administered in olive oil (16.7 μl/g of body weight) by intragastric tube with an intubation needle. Blood samples were obtained from the tail before and 1 h after [^{125}I]BMIPP administration. [^{125}I]BMIPP radioactivity was measured with a γ counter.

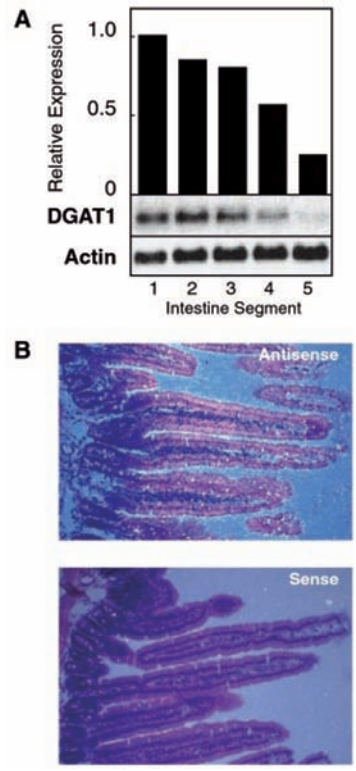

FIG. 1. **DGAT1 gene expression in mouse small intestine.** *A*, *Dgat1* mRNA (2.0-kb transcript) is present in all regions of the small intestine. RNA samples from five equal-length sections of small intestine were analyzed by Northern blotting (section 1 most proximal and section 5 most distal to the stomach). *B*, *in situ* hybridization demonstrating expression of *Dgat1* mRNA in the intestinal villi (*top panel*). Specific hybridization was not detected by a sense probe control (*bottom panel*).

Histology and Ultrastructure—Tissues were fixed by cardiac perfusion with 1.5% glutaraldehyde, 4% polyvinylpyrrolidone, 0.05% calcium chloride, and 0.1 M sodium cacodylate, pH 7.4. Intestines were removed, divided into sections representing duodenum, jejunum, and ileum and put directly into fixative. For light microscopy, tissues were immersed in 2% osmium tetroxide in 0.1 M sodium phosphate, pH 7.4, to stain neutral lipids. Tissues were then dehydrated in ethanol, transitioned into propylene oxide, and embedded in Epon 812 (DuPont, Wilmington, DE). Sections were counterstained in toluidine blue. For electron microscopy, the tissues were stained for lipid by the imidazole-buffered osmium tetroxide procedure (17), stained *en bloc* in 2% aqueous uranyl acetate for 1 h at 4 °C, and embedded in Epon 812. Ultrathin sections were stained for 5 min with 0.8% lead citrate and photographed with an electron microscope (Siemens Elmiskop 101, Siemens/CTI Corp., Knoxville, TN).

Tissue Lipid Analysis—Intestines were divided into five equal sections, and homogenates from section 2 as described above were prepared. Lipids were extracted from homogenates (250 μg of protein) with chloroform:methanol (2:1 v:v), dried under nitrogen, and separated by thin-layer chromatography (TLC) in hexane:ethyl ether:acetic acid (80:20:1 v:v:v). Lipids were visualized by immersing the TLC plate in a solution of cupric sulfate (10%) and phosphoric acid (8%) followed by heating at 180 °C for 15 min. The triacylglycerol and diacylglycerol bands were quantified by densitometry.

Enzyme Activity Assays—DGAT and diacylglycerol transacylase activities were measured in tissue homogenates (40–50 μg of protein) under apparent V_{max} conditions as described (6, 8), except that diacylglycerol was added in an acetone solution (18). In brief, DGAT assays measured the incorporation of [^{14}C]oleoyl CoA (specific activity: ~20,000 dpm/nmol) into triacylglycerols in a 5-min assay. The reaction mix contained 0.4 mM diacylglycerol and 25 μM oleoyl-CoA as substrates and 5 mM $MgCl_2$. Diacylglycerol transacylase assays measured the incorporation of [^{14}C]diacylglycerol (specific activity: 20 μCi/μmol)

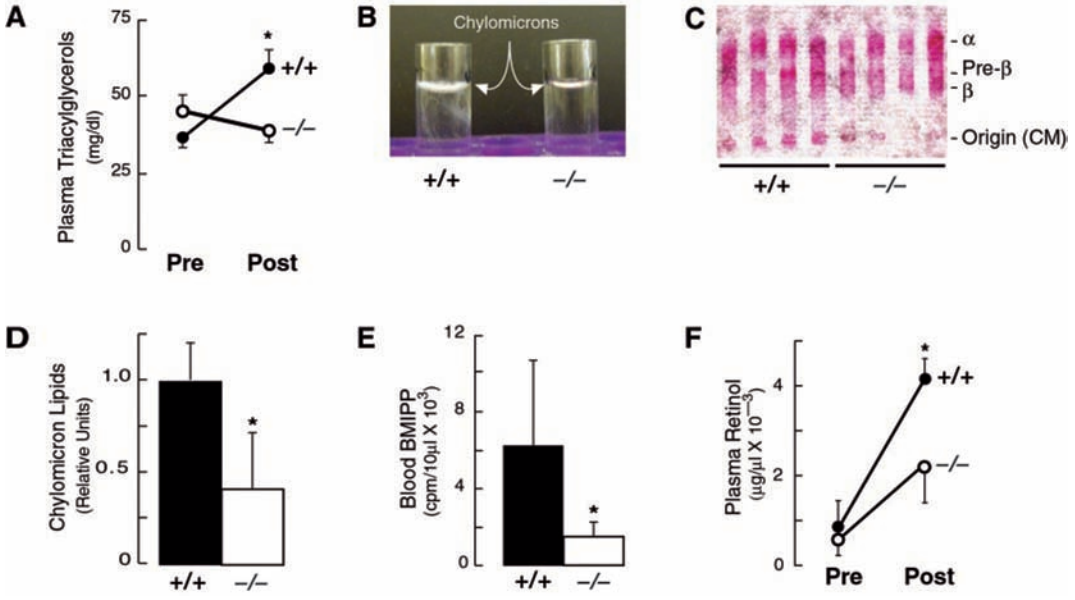

FIG. 2. **Diminished chylomicronemia after an acute dietary lipid challenge.** *A*, plasma triacylglycerol levels before (*Pre*) and 1 h after (*Post*) an intragastric oil bolus. Levels were similar in wild-type and *Dgat1*$^{-/-}$ mice before the bolus ($p = 0.18$, $n = 11$ mice of each genotype). After the bolus, wild-type mice had significantly higher plasma triacylglycerol concentrations (*, $p = 0.01$), whereas *Dgat1*$^{-/-}$ mice did not ($p = 0.15$). Data are means ± S.E. *B*, appearance of postabsorptive plasma after ultracentrifugation. Pooled plasma samples ($n = 4$ mice) obtained 1 h after an intragastric oil bolus were subjected to ultracentrifugation ($d < 1.006$). Note the visible chylomicrons at the top of the tube in wild-type mice. The band at the *top* of the tube in *Dgat1*$^{-/-}$ mice is due to the meniscus (and a small amount of chylomicrons). *C*, agarose gel electrophoresis of plasma samples obtained 1 h after an intragastric oil bolus. The gel was stained for lipid with Fat Red 7B. Each lane contains plasma from one mouse. The experiment was repeated three times with similar results. Chylomicrons are at the origin, very low density lipoproteins in the pre-β band, low density lipoproteins in the β band, and high density lipoproteins in the α band. *D*, reduced chylomicrons in postabsorptive plasma of *Dgat1*$^{-/-}$ mice analyzed by agarose gel electrophoresis. The lipid staining in the chylomicron region of plasma samples in "*C*" was quantified by densitometry. *, $p = 0.04$ *versus* wild-type mice, $n = 4$ mice of each genotype. *E*, diminished BMIPP in postabsorptive plasma of *Dgat1*$^{-/-}$ mice. BMIPP in an oil bolus was administered intragastrically, and radioactivity was measured in the blood 1 h later. *, $p = 0.045$ *versus* wild-type mice, $n = 5$ mice of each genotype. *F*, reduced retinol palmitate absorption in *Dgat1*$^{-/-}$ mice. Retinol palmitate in corn oil was administered intragastrically, and retinol was measured in the plasma 2 h later (*Post*). *, $p = 0.04$ *versus* wild-type mice, $n = 3$ mice of each genotype.

(American Radiolabeled) into triacylglycerols in a 10-min assay. The final concentration of diacylglycerol was 0.25 mM. At the conclusion of both assays, lipids were extracted with chloroform:methanol (2:1 v:v), dried under nitrogen, and separated by TLC in hexane:ethyl ether: acetic acid (80:20:1 v:v:v). Triacylglycerol bands were scraped, and radioactivity was measured by scintillation counting.

Statistical Analyses—Data are presented as mean ± S.D. except where indicated. For parametric data, means were compared with a *t* test or paired *t* test. For nonparametric data, a Mann-Whitney rank-sum test was used.

RESULTS

Dgat1 Gene Expression in Small Intestine—Dgat1 gene expression in the mouse small intestine was examined by Northern blots of RNA isolated from intestinal sections of wild-type mice. *Dgat1* was expressed in all sections; expression levels were highest in the most proximal region and were progressively lower in the more distal regions (Fig. 1*A*). We also examined *Dgat1* mRNA expression in the duodenum by *in situ* hybridization. High levels of *Dgat1* mRNA transcripts were found in enterocytes along the villi and in villus tips (Fig. 1*B*).

Normal Quantitative Dietary Fat Absorption in Dgat1$^{-/-}$ *Mice—Dgat1*$^{-/-}$ mice and wild-type mice fed either a low or high fat diet excrete similar amounts of fecal fat, suggesting they are able to absorb dietary fat normally (9). To better quantify dietary fat absorption in *Dgat1*$^{-/-}$ mice, we measured their ability to absorb [^{14}C]oleic acid administered intragastrically in an oil bolus. *Dgat1*$^{-/-}$ mice, like wild-type mice, absorbed nearly 100% of the radiolabeled tracer (not shown). Additionally, the plasma levels of α-tocopherol, a fat-soluble vitamin whose levels reflect dietary fat absorption (14), were similar in *Dgat1*$^{-/-}$ mice and wild-type mice (5.72 ± 0.51 *versus* 5.84 ± 0.77 μM, respectively; $n = 5$ mice, $p = 0.37$). These results all indicate that DGAT1 is not essential for the quantitative absorption of dietary fat.

Diminished Chylomicronemia after an Acute Dietary Lipid Challenge—Plasma triacylglycerol concentrations after a 4-h fast were similar in wild-type and *Dgat1*$^{-/-}$ mice (Fig. 2*A*), as reported previously (9). However, 1 h after the intragastric administration of a bolus of corn oil plasma triacylglycerol concentrations increased in wild-type mice but not in *Dgat1*$^{-/-}$ mice (Fig. 2*A*). This difference was attributable to differences in the amount of plasma chylomicrons, as demonstrated by analyzing plasma by ultracentrifugation (Fig. 2*B*) and agarose gel electrophoresis (Fig. 2*C*). Lipid staining in the chylomicron region of agarose gels was ~60% lower in *Dgat1*$^{-/-}$ mice than in wild-type mice (Fig. 2*D*). In addition, 1 h after intragastric administration, plasma levels of BMIPP, a 3-methyl-branched fatty acid analog that is only slowly catabolized by β-oxidation (16), were 75% lower in *Dgat1*$^{-/-}$ mice than in wild-type mice (Fig. 2*E*). Retinol palmitate absorption, an indicator of chylomicron synthesis and secretion (14), was also ~50% lower in *Dgat1*$^{-/-}$ mice than in wild-type mice 2 h after administration (Fig. 2*F*). These results indicate that *Dgat1*$^{-/-}$ mice have reduced postabsorptive chylomicronemia in response to an acute lipid challenge.

Abnormal Accumulation of Neutral-lipid Droplets in the Cytoplasm of Enterocytes in Dgat1$^{-/-}$ *Mice*—To better understand how DGAT1 deficiency affects lipid metabolism in enterocytes, we examined histological sections from the small intestines of wild-type and *Dgat1*$^{-/-}$ mice chronically fed a chow or high fat diet. In chow-fed mice, the microscopic appearance of intestines was normal for wild-type and *Dgat1*$^{-/-}$ mice (not shown). However, in *Dgat1*$^{-/-}$, but not wild-type mice fed a diet rich in milk fat or corn oil, the enterocytes in the duodenum and jejunum had large, neutral lipid–staining droplets in

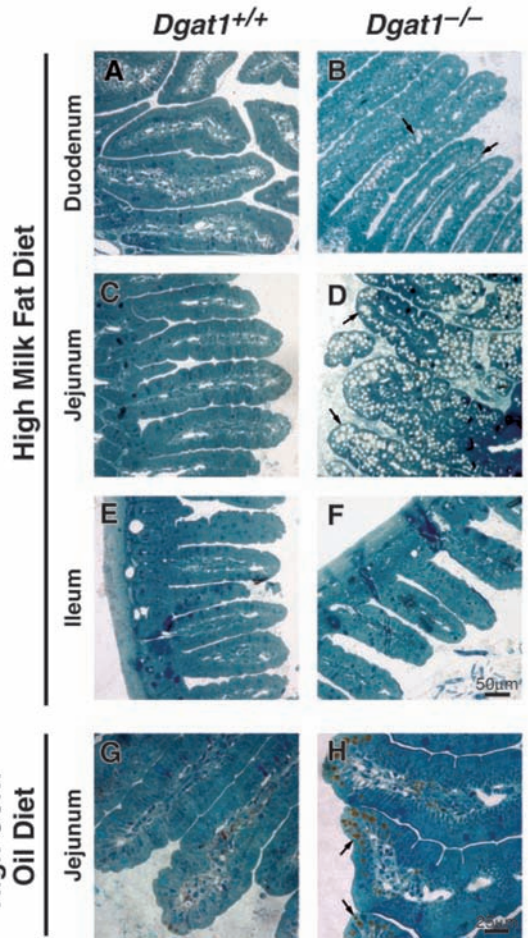

FIG. 3. **Accumulation of neutral lipid-staining droplets in the cytoplasm of enterocytes from *Dgat1*$^{-/-}$ mice fed a high fat diet for 3 weeks.** Large neutral-lipid droplets (stained *green-brown* by osmium tetroxide, *arrows*) were present in the cytoplasm of enterocytes in the duodenum and jejunum in *Dgat1*$^{-/-}$ but not wild-type intestine, regardless of whether the mice were fed a diet rich in milk fat or corn oil. Note the more intense osmium tetroxide staining of lipids in *Dgat1*$^{-/-}$ intestine of mice fed a diet rich in corn oil (*H*) than in mice fed a diet rich in milk fat (*B* and *D*). This likely reflects the dietary fat composition. Corn oil is rich in polyunsaturated fatty acids, whereas milk fat is rich in saturated fatty acids, and osmium tetroxide stains double bonds in tissue triglycerides. Neutral lipid-staining droplets were not present in enterocytes of the ileum in mice of either genotype.

the cytoplasm (Fig. 3). Lipid droplets were not found in enterocytes in the ileum of *Dgat1*$^{-/-}$ mice. The accumulation of lipid droplets in duodenal and jejunal enterocytes was much more prominent in *Dgat1*$^{-/-}$ mice fed a diet rich in milk fat (containing primarily saturated fatty acids) than in those fed a diet rich in corn oil (containing primarily polyunsaturated fatty acids). After an overnight fast, lipid droplets were absent in enterocytes from both wild-type and *Dgat1*$^{-/-}$ mice (not shown).

To examine the composition of the lipid droplets in *Dgat1*$^{-/-}$ enterocytes, we extracted lipids from sections of the proximal intestine of mice fed a diet rich in milk fat and analyzed the lipid extracts by TLC (Fig. 4*A*). Triacylglycerol levels were more than 2-fold higher, and diacylglycerol levels were ~50% higher, in *Dgat1*$^{-/-}$ intestines than in wild-type intestines (Fig. 4*B*).

DGAT1 Is Not Essential for Chylomicron Synthesis—To determine whether *Dgat1*$^{-/-}$ mice synthesize chylomicron-sized particles, we examined the ultrastructure of enterocytes from wild-type and *Dgat1*$^{-/-}$ mice fed a diet rich in corn oil. In both wild-type and *Dgat1*$^{-/-}$ mice, chylomicron-sized particles were observed within the luminal spaces of the endoplasmic reticulum and Golgi apparatus (Figs. 5 and 6). In addition, some enterocytes from *Dgat1*$^{-/-}$ mice had a single, large, supranuclear lipid droplet in their cytoplasm (often several microns in diameter), whereas wild-type mice did not (Fig. 5).

Alternative Mechanisms for Triacylglycerol Synthesis in the Small Intestine of Dgat1$^{-/-}$ *Mice*—[^{14}C]Oleic acid (in corn oil) infused into the lumen of the dudoenum in *Dgat1*$^{-/-}$ mice was incorporated into triacylglycerols by enterocytes within minutes (not shown). We therefore searched for possible alternative mechanisms of triacylglcyerol synthesis in *Dgat1*$^{-/-}$ enterocytes. We analyzed the expression of *Dgat2*, a newly identified DGAT gene that is expressed in the small intestine (7), in segments of the small intestine. As was the case for *Dgat1*, *Dgat2* was expressed in all intestinal segments, with higher expression levels in the more proximal regions and lower levels in the distal regions (Fig. 7*A*). *Dgat2* expression levels were similar in intestines of *Dgat1*$^{-/-}$ mice and wild-type mice (not shown).

We were unable to examine DGAT1 and DGAT2 protein levels due to the lack of suitable antibodies. However, we measured DGAT activity in homogenates from intestinal segments of wild-type and *Dgat1*$^{-/-}$ mice using assay conditions (5 mM $MgCl_2$ in the assay mix) that detect both DGAT1 and DGAT2 activities (7). DGAT activity levels in wild-type intestines were highest in the proximal sections and were ~50% lower in the most distal section (Fig. 7*B*). In *Dgat1*$^{-/-}$ intestines, DGAT activity levels (presumably reflecting DGAT2 activity) in all sections were ~10–15% of the levels in wild-type mice.

An additional acyl-CoA–independent mechanism for synthesizing triacylglycerol from diacylglycerol may involve diacylglycerol transacylase (8). In wild-type mice, we found diacylglycerol transacylase activity in homogenates at high levels along the length of the intestine, with 2-fold higher activity levels in the most proximal section than in other sections (Fig. 7*C*). *Dgat1*$^{-/-}$ mice had similar levels of diacylglycerol transacylase activity.

DISCUSSION

DGAT1 is highly expressed in the small intestine, where it is believed to function in triacylglycerol synthesis and absorption. In this study, we analyzed intestinal triacylglycerol absorption and the ability to synthesize chylomicron-sized lipoprotein particles in *Dgat1*$^{-/-}$ mice. DGAT1 was not essential for quantitative dietary triacylglycerol absorption or for chylomicron synthesis. However, *Dgat1*$^{-/-}$ mice had reduced postabsorptive chylomicronemia and accumulated neutral-lipid droplets in the cytoplasm of enterocytes when chronically fed a high fat diet, suggesting a reduced rate of triacylglycerol absorption. At least two other enzymes, DGAT2 and diacylglycerol transacylase, appear to catalyze triacylglycerol synthesis in mouse intestine and help to compensate for the absence of DGAT1.

DGAT activity is present along the length of the small intestine, with the highest levels in the proximal region (3, 4). We found that the *Dgat1* mRNA expression pattern in the small intestine was similar to that of DGAT activity and that DGAT1 appears to account for the majority of intestinal DGAT activity as measured by *in vitro* assays. *Dgat1* mRNA was present at high levels in intestinal villus tips, consistent with reports of higher levels of DGAT activity in villus tips than in crypt cells (4). *Dgat2* mRNA expression was also higher in proximal than in distal segments of the small intestine.

Although DGAT1 was not essential for quantitative dietary triacylglycerol absorption, DGAT1 deficiency had significant effects on intestinal triacylglycerol metabolism when the dietary load of fat was high. For example, *Dgat1*$^{-/-}$ mice had reduced postabsorptive chylomicronemia after the administra-

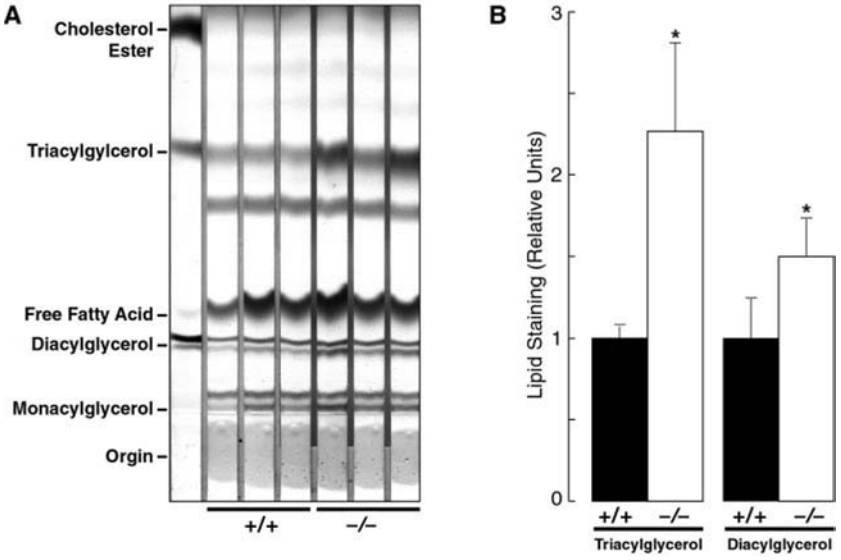

FIG. 4. **Accumulation of triacylglycerol and diacylglycerol in the small intestine of *Dgat1*$^{-/-}$ mice fed a high milk fat diet for 3 weeks.** *A*, small intestines from wild-type and *Dgat1*$^{-/-}$ mice were divided into five equal-length sections, and homogenates were prepared from section 2. Lipids were extracted and separated by TLC. *B*, triacylglycerol and diacylglycerol bands were quantified by densitometry, and results were normalized for the protein content of the samples. *, $p = 0.0495$ *versus* wild-type, $n = 3$ mice of each genotype.

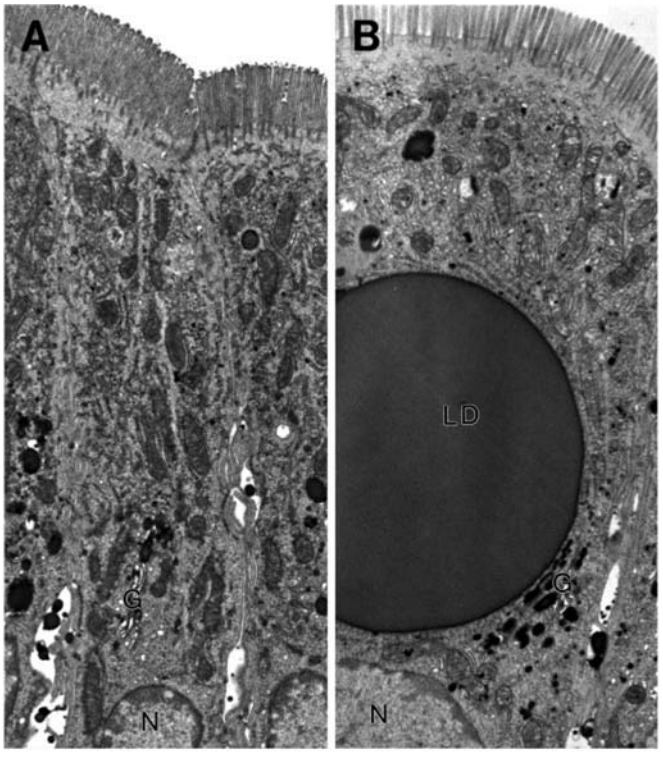

FIG. 5. **Ultrastructural analysis of *Dgat1*$^{-/-}$ enterocytes.** Low power electron micrographs (×6200) show a single, large neutral-lipid droplet (*LD*) in the cytoplasm typically above the Golgi apparatus (*G*) and nucleus (*N*) of some *Dgat1*$^{-/-}$ (*B*) but not wild-type enterocytes (*A*) from the duodenum of mice fed a diet rich in corn oil for 3 weeks. Chylomicron-sized particles are present in the Golgi apparatus of both wild-type and *Dgat1*$^{-/-}$ mice.

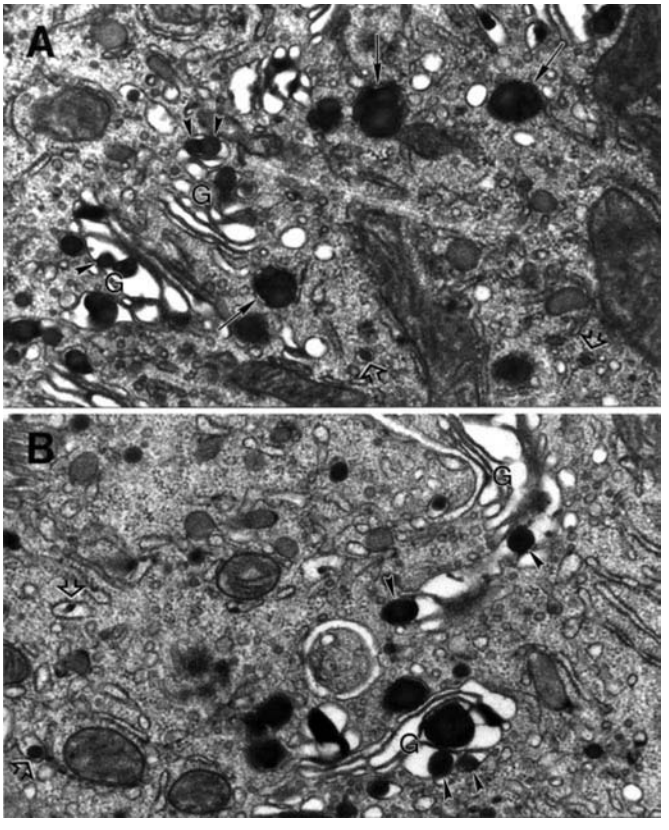

FIG. 6. **Chylomicron-sized particles in *Dgat1*$^{-/-}$ enterocytes.** Higher power electron micrographs (×19,000) show the presence of chylomicron-sized particles within the endoplasmic reticulum cisternae (*open arrows*), within membrane compartments of the Golgi apparatus (*G*) (*arrowheads*), and sometimes clustered in secretory vesicles (*arrows*) of both wild-type (*A*) and *Dgat1*$^{-/-}$ enterocytes (*B*) from the duodenum of mice fed a diet rich in corn oil for 3 weeks.

tion of a bolus of dietary triacylglycerol. In addition, lipid droplets accumulated in *Dgat1*$^{-/-}$ enterocytes when the mice were chronically fed a high fat diet. These data suggest that the capacity for residual triacylglycerol synthesis in the absence of DGAT1 is adequate when dietary fat levels are low but is overwhelmed by high levels of dietary fat. As a result, movement of fat through the enterocyte is delayed, reducing the rate at which chylomicrons enter the plasma. The precise role of DGAT1 in this process remains unknown. Although enterocytes normally do not store large amounts of lipid in their cytoplasm, newly synthesized triacylglycerol may first have to enter a storage pool before subsequent hydrolysis and resynthesis for chylomicron assembly (19). DGAT1 may help synthesize triacylglycerol for a specific step in this sequence, and the absence of DGAT1 may therefore decrease throughput. A better understanding of the enzymes involved in triacylglycerol synthesis and their cellular location in enterocytes will be needed to determine the precise function of DGAT1. Nevertheless, our data indicate that DGAT1 is not directly required for the assembly of triacylglycerol-rich, chylomicron-sized particles.

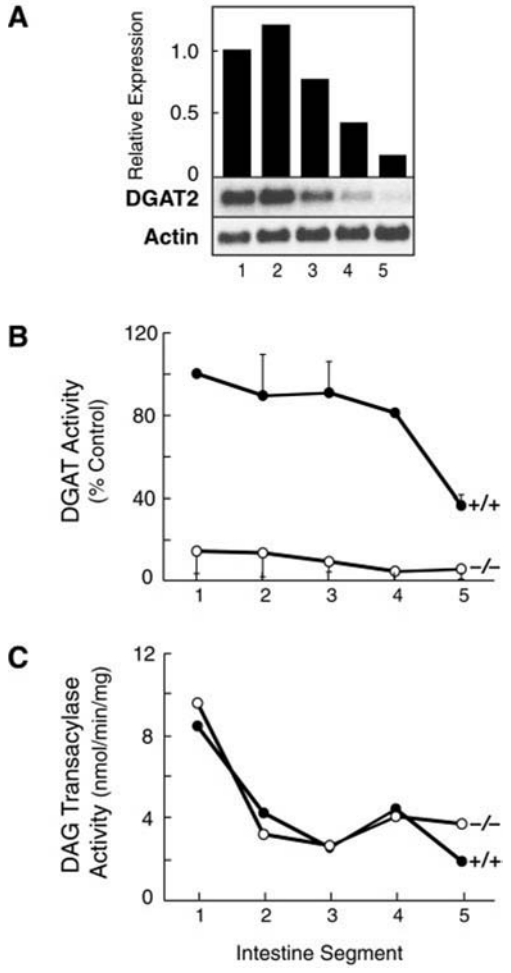

FIG. 7. **Alternative mechanisms for triacylglycerol synthesis in the small intestine of *Dgat1*$^{-/-}$ mice.** *A*, Northern analysis of mRNA shows *Dgat2* expression (2.4-kb transcript) in all regions of the small intestine. *B*, DGAT activity in sections of the small intestine of wild-type and *Dgat1*$^{-/-}$ *mice*. DGAT activity in membranes was measured as described under "Experimental Procedures." Data are expressed as percent of DGAT activity found in intestine section 1 of wild-type mice (3876 ± 893 pmol/min/mg of protein); $n = 3$ mice of each genotype. *C*, diacylglcyerol transacylase activity in sections of the small intestine of wild-type and *Dgat1*$^{-/-}$ mice. Diacylglcyerol transacylase activity was measured as described under "Experimental Procedures." Data shown are means of two separate experiments.

Our results illustrate the remarkable capacity of the small intestine to absorb dietary triacylglycerols, even in the absence of a key enzyme. Cytosolic lipid droplets were prominent in the duodenum and jejunum but not in the ileum of *Dgat1*$^{-/-}$ mice fed a high fat diet, suggesting that compensatory mechanisms were sufficient to prevent dietary fat from reaching the large intestine. The excess capacity for triacylglycerol absorption probably involves redundant mechanisms for synthesizing triacylglycerols and the great length and surface area of the small intestine. Indeed, the ileum can fully compensate to facilitate triacylglycerol absorption in the absence of the jejunum (20). The high efficiency and capacity for triacylglycerol absorption in the small intestine may reflect an evolutionary pressure to maximize the absorption of energy-rich fat during times of starvation.

The histologic examination of *Dgat1*$^{-/-}$ intestines also revealed that feeding a diet rich in saturated fat to *Dgat1*$^{-/-}$ mice resulted in a greater accumulation of lipid droplets in the intestine than a diet rich in polyunsaturated fatty acids. This suggests that saturated fatty acids may be better substrates than polyunsaturated fattty acids for DGAT1. However, *in vitro* studies in insect cells demonstrated that fatty acids of different saturation and chain length are used equally well as substrates by DGAT1 and DGAT2 (7). Regardless, the data suggest that saturated fatty acids enter a chylomicron synthesis pathway in enterocytes that is relatively specific for DGAT1.

Our results indicate that at least two enzymes, DGAT2 and diacylglycerol transacylase, contribute to triacylglycerol synthesis in the absence of DGAT1. The relative importance of DGAT2 is currently unclear. Although *Dgat2* was expressed throughout the small intestine, residual DGAT activity was low (~10–15%) in *Dgat1*$^{-/-}$ intestines. We suspect that the *in vitro* assay measurements of DGAT2 activity may underestimate the contribution of DGAT2 to intestinal triacylglycerol synthesis. This possiblility is illustrated by studies of *Dgat1*$^{-/-}$ adipocytes, which accumulate triacylglycerols to levels ~50% of those in wild-type mice even though DGAT activity is virtually absent in *Dgat1*$^{-/-}$ adipose tissue membranes (7). The high levels of diacylglycerol transacylase activity indicate that this enzyme may also play a prominent role in triacylglycerol synthesis in mouse intestine. Defining the relative roles of DGAT2 and diacylglycerol transacylase in intestinal triacylglycerol synthesis and absorption is an important issue for future research.

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