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## A Specific Role for *Dgat1* in Hepatic Steatosis Due to Exogenous Fatty Acids

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### Abstract

Nonalcoholic fatty liver disease, characterized by accumulation of triacylglycerols (TG) and other lipids in the liver, often accompanies obesity and is a risk factor for nonalcoholic steatohepatitis and fibrosis. To treat or prevent fatty liver, a thorough understanding of hepatic fatty acid and TG metabolism is crucial. To investigate the role of acyl CoA:diacylglycerol acyltransferase 1 (DGAT1), a key enzyme of TG synthesis, in fatty liver development, we studied mice with global and liver-specific knockout of *Dgat1*. DGAT1 was required for hepatic steatosis induced by high-fat diet and prolonged fasting, which are both characterized by delivery of exogenous fatty acids to the liver. Studies in primary hepatocytes showed that DGAT1 deficiency protected against hepatic steatosis by reducing synthesis and increasing the oxidation of fatty acids. In contrast, lipodystrophy (*aP2-SREBP-1c436*) and liver X receptor activation (T0901317), which increase *de novo* fatty acid synthesis in liver, caused steatosis independently of DGAT1. Pharmacologic inhibition of *Dgat1* with antisense oligonucleotides protected against fatty liver induced by a high-fat diet. In conclusion, our findings identify a specific role for hepatic DGAT1 in esterification of exogenous fatty acids and indicate that DGAT1 contributes to hepatic steatosis induced by this mechanism.

### Keywords

Triglyceride synthesis; obesity; diabetes; DGAT; fatty liver

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Nonalcoholic fatty liver disease (NAFLD) is characterized by lipid accumulation in hepatocytes of people who consume little to no alcohol (1–3). Chronic lipid accumulation in the liver (hepatic steatosis) is a risk factor for nonalcoholic steatohepatitis, an inflammatory condition in some patients with fatty liver (4). NAFLD is the most common cause of abnormal liver enzyme tests (5), is associated with obesity and insulin resistance, and is

increasing in prevalence, affecting ~30 million adults in the U.S., making it the most common liver disorder (2, 6). NAFLD portends epidemic problems for public health, and a better understanding of the pathways that regulate lipid accumulation in the liver is crucial for developing effective therapies for hepatic steatosis.

Lipids that accumulate in hepatic steatosis are mainly triacylglycerols (TGs). After adipose tissue, the liver has perhaps the largest capacity to synthesize and store TGs (7). TGs are products of the glycerol phosphate synthesis pathway, in which fatty acyl moieties are joined to glycerol via ester bonds (8, 9). FAs in the liver may come from exogenous sources (e.g., dietary fat or mobilization from white adipose tissue (WAT) during fasting) or from endogenous *de novo* synthesis promoted by leptin deficiency or high levels of circulating insulin and glucose (1, 10–12).

The final step in TG synthesis is catalyzed by DGAT enzymes. Mammals have two DGAT enzymes that are members of distinct gene families (13, 14). Both are expressed widely in tissues and in the livers of mice and humans (13, 15). Increased levels of DGAT1 mRNA, in particular, occur in human livers with NAFLD (16), underscoring the importance of defining the role of DGAT1 in this tissue. Mice lacking *Dgat1* (*Dgat1*<sup>-/-</sup>) are viable, have reduced tissue TG levels, exhibit increased sensitivity to insulin and leptin, and are protected against diet-induced obesity through increased energy expenditure (17, 18). However, DGAT1's function in hepatic steatosis has not been fully explored.

To investigate the role of DGAT1 in hepatic steatosis, we studied mice with global (17) and liver-specific knockout of *Dgat1* under conditions that promote hepatic steatosis. These included a high-fat diet, fasting (in which lipids are mobilized from the WAT to the liver), and two conditions in which endogenous FA synthesis is greatly increased—genetically induced lipodystrophy (19) and treatment with the liver X receptor (LXR) agonist T0901317 (20). Relevant to clinical therapies, we also determined whether knockdown of *Dgat1* expression with antisense oligonucleotides (ASO) protects against diet-induced hepatic steatosis.

## Experimental Procedures

### Mice

*Dgat1*<sup>-/-</sup> and wild-type mice (C57BL/6J background) were genotyped as described (17). Mice were housed in a pathogen-free barrier facility (12-h light/12-h dark cycle) and fed chow (5053 PicoLab Diet; Purina) or a high-fat diet (20% milk fat, 0.2% cholesterol by weight; TD 01064 Harlan-Teklad). *Dgat1*<sup>flox/flox</sup> mice were generated as described (Supplementary Methods). During fasting, mice had access to water. *Dgat1*<sup>-/-</sup> mice were crossed with *aP2-SREBP-1c436* transgenic mice (Jackson Laboratory; 50% C57BL/6J, 50% SJL). *Dgat1*<sup>+/-</sup> *aP2-SREBP-1c436* mice (75% C57BL/6J, 25% SJL) were crossed with *Dgat1*<sup>-/-</sup> (C57BL/6J) males to generate *Dgat1*<sup>-/-</sup> *aP2-SREBP-1c436* mice (87.5% C57BL/6J, 12.5% SJL). All experiments were approved by the Committee on Animal Research, University of California, San Francisco.

### Histological Analyses

See Supplementary Methods.

### Lipid Analyses

Livers were homogenized in buffer containing 50 mM Tris-HCl, pH 7.4, and 250 mM sucrose with complete protease inhibitor (Roche). Lipids were extracted with

chloroform:methanol (2:1) and separated as described (21). TG bands were identified, scraped, and quantified spectrophotometrically (22).

### Adenoviruses

*Cre*-, *LacZ*-, and *GFP*-expressing adenoviruses (Vector Development Laboratory, Baylor College;  $2 \times 10^{11}$  particles in 0.2 ml of PBS) were injected into a jugular vein (23) of *Dgat1<sup>flox/flox</sup>* mice (16–20 weeks old). After 4 weeks, mice were placed on a high-fat diet for 3 weeks or fasted for 20 h.

### ASOs

Control and *Dgat1* ASO (Isis Pharmaceuticals) with an 2'-O-(2-methoxy)-ethyl modification at the first and last five bases (24) were injected (50 mg/kg intraperitoneally) twice weekly for 5 weeks.

### DGAT Activity Assays

Livers were homogenized in Buffer A (50 mM Tris-HCl, pH 7.4, 250 mM sucrose) with proteinase inhibitors (Roche Diagnostic). To prepare microsomes, homogenates were centrifuged three times at 4°C (600×g for 5 min, 10,000×g for 10 min, 100,000×g for 1 h); after each centrifugation, pellets were resuspended in Buffer A. DGAT assays were performed with microsome proteins (100 µg) in mix containing 100 mM MgCl<sub>2</sub>, 1.25 mg/ml bovine serum albumin, 200 µM 1,2-dioleoyl-*sn*-glycerol (Sigma-Aldrich) in acetone, and 25 µM [<sup>14</sup>C]oleoyl-CoA (53.0 mCi/mmol). After 10 min at 37°C, lipids were extracted with chloroform:methanol (2:1, v:v) and separated on G-60 TLC plates with hexane:ethyl ether:acetic acid (80:20:1, v:v:v). TG bands were scraped, and radioactivity was measured by scintillation counting.

### FA Oxidation Measurement

Hepatocytes were incubated with 3 ml of DMEM containing 200 µM [9,10-<sup>3</sup>H]oleic acid (10 µCi/ml) (Amersham) conjugated to 0.2% bovine serum albumin for 2 h. The medium was saved, cells were scraped with 1 ml of 0.1 N NaOH and 1 ml of water, and protein concentrations determined by Dc protein assay (Biorad). Medium (100 µl) was placed in a microcentrifuge tube, which was placed in a 20-ml scintillation vial containing 500 µl of water and sealed and heated at 50°C for ~18 h for [<sup>3</sup>H]H<sub>2</sub>O to equilibrate. Equilibrium efficiency was determined by similar analysis of 100 µl of 0.1 µCi/µl of [<sup>3</sup>H]H<sub>2</sub>O (~65%) or [9,10-<sup>3</sup>H]oleic acid (~0.5%). After cooling, microcentrifuge tubes removed, scintillation fluid (aqueous/nonaqueous) was added, and [<sup>3</sup>H]H<sub>2</sub>O was measured with a scintillation counter.

### RNA Extraction and Real-Time PCR

RNA was extracted from livers with RNA STAT-60 (Tel-Test) and treated with DNase (Ambion). cDNA was synthesized from RNA (5 µg) with Superscript II reverse transcriptase and random hexamers (Invitrogen). Real-time PCR primers (Supplementary Table 2) were selected with Primer Express (version 1.5; Applied Biosystems). Two-step RT-PCR were performed with Sybrgreen (Applied Biosystems) and an ABI 9600.

### Statistical Analyses

Values are mean ± SEM. Means were compared by *t*-test or ANOVA and Student-Newman-Keuls test.

## Results

### Hepatic DGAT1 Deficiency Protects against High-fat-diet-induced Fatty Liver

Mice were fed a High-fat diet for 3 weeks. *Dgat1*<sup>+/+</sup> livers were pale and stained positively for lipids (Fig. 1A). Lipid staining was reduced in *Dgat1*<sup>-/-</sup> livers, consistent with prolonged (32 weeks) high-fat feeding (17). Sirius Red staining showed similar amounts of collagen in *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> livers (not shown). Hepatic TG levels were similar in chow-fed *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> mice but ~80% lower in *Dgat1*<sup>-/-</sup> mice after high-fat feeding (Fig. 1B). All classes of FAs in TG were reduced. FA synthesis was increased in hepatocytes from *Dgat1*<sup>+/+</sup> mice fed a high-fat diet (Fig. 1C), consistent with reported effects (25), but reduced in hepatocytes from *Dgat1*<sup>-/-</sup> mice, as were mRNA levels of the lipogenic transcription factors *Srebp1c* and its targets FA synthase (*Fasn*) and stearoyl-CoA desaturase 1 (*Scd1*), and carbohydrate response element binding protein (*Chrebp*) and its target liver-pyruvate kinase (*L-pk*) (Fig. 1D). This may reflect the lower serum insulin levels found in *Dgat1*<sup>-/-</sup> than *Dgat1*<sup>+/+</sup> mice ( $0.7 \pm 0.1$  vs.  $1.6 \pm 0.3$  ng/ml,  $n=4-5$ /genotype,  $P<0.05$ ). Protection was associated with increased FA oxidation in hepatocytes from fat-fed *Dgat1*<sup>-/-</sup> mice (Fig. 1E); no differences were found in chow-fed mice. Consistent with increased FA oxidation, AMP-activated kinase, which induces hepatic FA oxidation (26), was activated in livers of fat-fed *Dgat1*<sup>-/-</sup> mice fed (Supplementary Fig. 1). mRNA levels of several genes involved in FA oxidation genes were similar in *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> mice (Fig. 1F), suggesting increased substrate flux.

Because *Dgat1* is widely expressed in murine tissues, protection of *Dgat1*<sup>-/-</sup> mice may reflect loss of DGAT1 in liver or other tissues affecting hepatic TG balance (27–29). In mice with adenovirus-mediated knockdown of *Dgat1* in liver (LivD1KO mice) (Supplementary Fig. 1), hepatic *Dgat1* mRNA levels were ~80% lower than in controls, and the knockdown was specific (Fig. 2A). FA synthesis and oxidation genes were similarly expressed in control and LivD1KO mice (Fig. 2A). DGAT activity was reduced by ~80% in livers of LivD1KO mice (Fig. 2B), or slightly less than in the global knockout (17), possibly because of residual activity in nonhepatocyte cells (30). After 3 weeks of high-fat feeding, LivD1KO livers were darker (Fig. 2C) and had ~50% lower hepatic TG levels (Fig. 2D), indicating some protection against steatosis.

### Hepatic DGAT1 Deficiency Protects against Fasting-induced Hepatic Steatosis

During fasting, free fatty acids mobilized from the adipose tissue to liver can be esterified to form TG, which can accumulate and cause steatosis (31). To explore the role of DGAT1 in this process, we measured hepatic *Dgat1* mRNA levels under different dietary conditions. Mice fasted for 16 h had almost 3-fold higher hepatic *Dgat1* mRNA levels than mice fed *ad libitum* or after refeeding (Fig. 3A). *Dgat2* mRNA levels were similar during these conditions.

We therefore examined hepatic steatosis in *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> mice fasted for 20 h. *Dgat1*<sup>-/-</sup> livers were darker than *Dgat1*<sup>+/+</sup> livers (Fig. 3B) and had ~70% lower TG content (Fig. 3C). Serum levels of the ketone  $\beta$ -hydroxybutyrate, a product of FA oxidation in the liver, were slightly elevated in fasted *Dgat1*<sup>-/-</sup> mice, although the differences did not reach significance ( $0.54 \pm 0.13$  versus  $0.32 \pm 0.07$  mM,  $n = 5$ /genotype,  $P < 0.07$ ; 8-h fast).

To determine if DGAT1 acts in a liver-specific manner during fasting, we examined steatosis in fasted mice. LivD1KO mice had ~75% lower hepatic *Dgat1* mRNA levels than controls but similar hepatic *Dgat2* mRNA levels and *Dgat1* mRNA levels in gastrocnemius muscle (data not shown) (Fig. 3D). No apparent changes were found in the lipid oxidizing transcription factor, peroxisome proliferator-activated receptor alpha (*Ppara*), as well as target genes such as carnitine-palmitoyl transferase 1 (*Cpt1*), aldehyde dehydrogenase 3

family member A2 (*Aldh3a2*), enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase (*Ehhadh*), and cytochrome P450, family 4, subfamily a, polypeptide 10 (*Cypa10*). LivD1KO mice had ~80% lower hepatic DGAT activity (Fig. 3E), darker livers (Fig. 3F), and ~80% lower hepatic TG levels (Fig. 3G), a reduction similar to that in *Dgat1*<sup>-/-</sup> mice. Further, *Dgat1*<sup>-/-</sup> hepatocytes had reduced capacity to synthesize TG when FA concentrations were above 250 μM (Fig. 3H).

### DGAT1 Deficiency Does Not Protect against Hepatic Steatosis Induced by Endogenous FA Synthesis

Congenital generalized lipodystrophy is characterized by paucity of adipose tissue, hyperinsulinemia, hyperglycemia, and hepatic steatosis. In *aP2-SREBP-1c436* transgenic mice, a model of this disorder, leptin levels are reduced, leading to upregulation of FA synthesis genes and hepatic steatosis (11, 12, 19).

To determine if hepatic steatosis in this model requires DGAT1, we generated *Dgat1*<sup>-/-</sup> *aP2-SREBP-1c436* mice. Hepatic *Srebp1c* expression was markedly elevated in *aP2-SREBP-1c436* and *Dgat1*<sup>-/-</sup> *aP2-SREBP-1c436* mice, as were mRNAs for SREBP1c targets and *Dgat2* mRNA levels (Fig. 4A). In both genotypes, hepatic TG content was similarly increased to higher levels than in *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> controls (Fig. 4B). Unlike livers of *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> mice, livers of *aP2-SREBP-1c436* and *Dgat1*<sup>-/-</sup> *aP2-SREBP-1c436* mice were pale, enlarged, and stained positively for lipids (Fig. 4C).

To test the hypothesis that DGAT1 is not required for hepatic steatosis from increased *de novo* FA synthesis, we treated *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> mice with an LXR agonist, T0901317, which activates FA synthesis in the liver and leads to hepatic steatosis (20). As expected, the mice had increased expression of *Fasn* and *Scd1* (Fig. 4D). Consistent with our hypothesis, *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> livers had similar degrees of steatosis after 2 weeks of treatment (Fig. 4E).

### Inhibiting DGAT1 Protects against High-fat-diet-induced Fatty Liver

Next, we assessed ASO-mediated knockdown of *Dgat1* as a treatment for fatty liver induced by a high-fat diet. *Dgat1* ASO reduced hepatic *Dgat1* mRNA levels without altering *Dgat2* expression (Fig. 5A). ASO pretreatment reduced by ~40% the increase in hepatic TG content after 1 week of high-fat diet (Fig. 5B).

## Discussion

This study shows that hepatic steatosis induced in mice by a high-fat diet or fasting, which promote hepatic uptake of exogenous FAs, required hepatic DGAT1. DGAT1 was not required for hepatic steatosis induced by lipodystrophy or LXR activation, which upregulate endogenous *de novo* FA synthesis. Thus, DGAT1 has a specific role in esterifying exogenous FAs. Pretreatment with DGAT1-specific ASO reduced hepatic TG content, suggesting that pharmacologic inhibition of DGAT1 may prevent hepatic steatosis induced by a high-fat diet.

DGAT1 deficiency reduced high-fat-diet-induced hepatic steatosis, a condition involving uptake of dietary FAs and the activation of FA synthesis (25). On a 3-week, high-fat diet, global DGAT1 deficiency reduced hepatic TG by ~80%. Hepatic deletion of *Dgat1* reduced hepatic TG by ~50%. The additional reduction with global *Dgat1* deficiency may reflect loss of DGAT1 activity in tissues, such as WAT (through endocrine effects) (32) or small intestine (33). Reduced FA synthesis may also have contributed to protection against steatosis. Lipogenesis is regulated by *Srebp1c*, an insulin-responsive transcription factor that is typically upregulated in livers of obese mice (11), and *Dgat1*<sup>-/-</sup> mice had reduced mRNA



levels of *Srebp1c* and FA synthesis enzymes. Reduced insulin levels in *Dgat1*<sup>-/-</sup> mice may explain their lower hepatic levels of *Srebp1c*, which may have resulted from the improved insulin sensitivity associated with *Dgat1* deficiency (18). Expression of these genes was not changed in LivD1KO mice, suggesting that the changes in lipogenesis in *Dgat1*<sup>-/-</sup> mice are indirect and may result from loss of DGAT1 in nonhepatic tissues.

Our results also show a role for hepatic DGAT1 in fasting. During a fast, hepatic glycogen content is depleted, and the liver switches to lipids as fuel. Lipolysis is activated in WAT (34–36), and free FAs are mobilized to the liver, where they are oxidized to yield ketones or re-esterified to TG for storage or secretion in VLDL (37, 38). In a previous study of mice, a 16-h fast increased hepatic *Dgat1* mRNA expression ~2.7-fold (37), suggesting a link between DGAT1 and steatosis in fasting. We confirmed this finding and, in studies of global and liver-specific *Dgat1* gene inactivation, demonstrated a functional requirement for hepatic DGAT1 during fasting since these mice were protected against steatosis. In the absence of hepatic DGAT1 expression, FAs entering the liver from WAT during fasting are likely oxidized, as suggested by the increase in circulating ketones in *Dgat1*<sup>-/-</sup> mice during fasting. The reduced steatosis did not appear to reflect increased hepatic VLDL secretion, since serum TG levels were lower in fasted *Dgat1*<sup>-/-</sup> mice (Supplementary Fig. 2). Finally, in primary hepatocytes, DGAT1 was required for the esterification of exogenous FA (palmitate) at high concentrations of substrate, further supporting a role for DGAT1 in esterifying exogenous FAs.

In contrast, steatosis caused by increased endogenous *de novo* FA synthesis was independent of DGAT1. In lipodystrophies, leptin is deficient, and FA synthesis is activated and contributes to TG accumulation in the liver (11, 12, 19, 39). *aP2-SREBP-1c436* and *Dgat1*<sup>-/-</sup> *aP2-SREBP-1c436* mice had similar degrees of hepatic steatosis, indicating that DGAT1 was not required for steatosis in this lipodystrophy model. Similarly, in *ob/ob* mice, in which leptin deficiency leads to upregulated FA synthesis, DGAT1 deficiency did not protect against hepatic TG accumulation (18) (H. Chen, R. Farese: unpublished observations). Hepatic steatosis induced by LXR activation of *de novo* FA synthesis was also similar in wild-type and *Dgat1*<sup>-/-</sup> mice. Similar results were found with *Dgat1* ASO treatment in mice administered LXR agonist T0901317 (Supplementary Fig. 3). In these situations of activated *de novo* lipogenesis, fatty acid oxidation is likely shutdown, and the effects of DGAT1 deficiency to activate this pathway may be obviated.

Our findings indicate that DGAT1 is not functionally linked to *de novo* FA synthesis and suggest that DGAT2 mediates TG synthesis in this situation. How the two enzymes couple to different sources of FAs is unknown. One possibility is functional compartmentalization, as DGAT2 physically associates with SCD1 (40), which desaturates newly synthesized FAs and functions in the *de novo* synthesis pathway (41). However, DGAT2 may not be exclusively linked to *de novo* synthesis, since ASO knockdown of *Dgat2* reduced steatosis in fat-fed mice (24). Evidence for compartmentalization of DGAT enzymes exists. In tung tree cells, DGAT1 and DGAT2 localize to different subdomains of the ER, where they may synthesize different pools of TG within the cell (42). Mammalian DGAT1 and DGAT2 also localize to distinct regions in hepatoma cells (43).

In contrast to our findings, Choi *et al.* (44) showed that *Dgat1* ASO did not block hepatic steatosis in rats fed a high-fat diet. However, the rats were treated with ASO after 3 days on a high-fat diet, whereas our mice were pretreated with ASO for 4 weeks before starting a 1-week high-fat diet. Moreover, our diet contained milk fat (57% saturated, 30% monounsaturated, and 3% polyunsaturated fat) and cholesterol, whereas theirs was rich in safflower oil (12% saturated, 13% monounsaturated, and 75% polyunsaturated fat). The composition of dietary fat can produce contrasting results in mice. For example, mice

lacking liver FA-binding protein are protected against hepatic steatosis when challenged with a diet rich in saturated but not polyunsaturated fat (45). In *db/db* mice, Yamaguchi *et al.* (30) also showed that *Dgat1* ASO reduced hepatic fibrosis, but did not protect against hepatic TG accumulation induced by a methionine choline–deficient diet, providing a role for DGAT1 in NASH. This finding is consistent with our finding that DGAT1 deficiency does not protect *ob/ob* (and *db/db*) mice against obesity, diabetes, and hepatic steatosis (18) (H. Chen, R. Farese: unpublished observations). This may be due in part to an induction of FA synthesis in *ob/ob* mice that drives TG synthesis mediated by DGAT2.

In conclusion, we showed that DGAT1 deficiency has a specific role in the development of steatosis due to exogenous FAs but not endogenous FA synthesis. Global and liver-specific inactivations of *Dgat1* and knockdown of *Dgat1* by ASO afforded protection from steatosis due to a high-fat diet. It remains to be determined how our findings in murine models translate to human disease. However, DGAT1 is expressed highly in human liver (15), and DGAT1 expression is increased in humans with NAFLD (16). Moreover, in a murine model of NASH, treatment with *Dgat1* ASO reduced hepatic fibrosis (30). Since high-fat diets are common in individuals with obesity and hepatic steatosis, DGAT1 inhibition may be a useful strategy for treating hepatic steatosis.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

<b>ASO</b>	antisense oligonucleotides
<b>DGAT1, acyl CoA</b>	diacylglycerol acyltransferase 1
<b>FA</b>	fatty acid
<b>LXR</b>	liver X receptor
<b>NAFLD</b>	nonalcoholic fatty liver disease
<b>TG</b>	triacylglycerols

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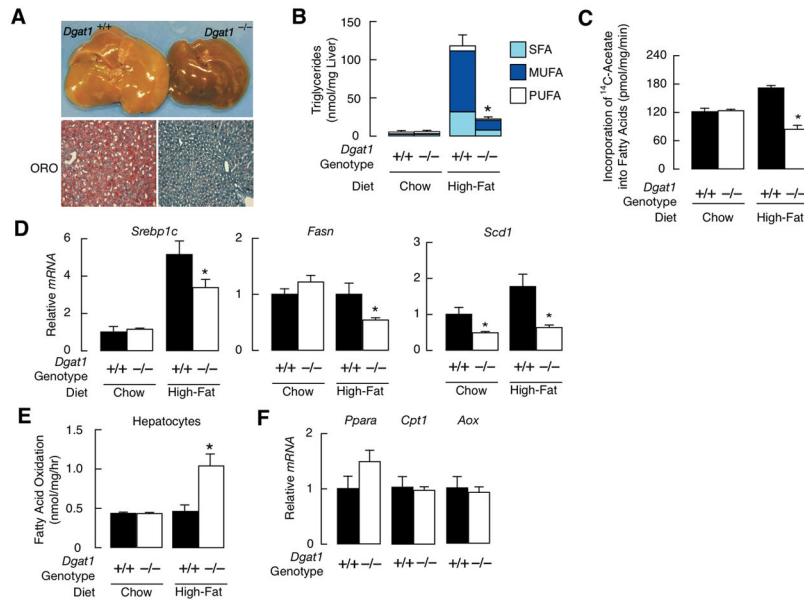
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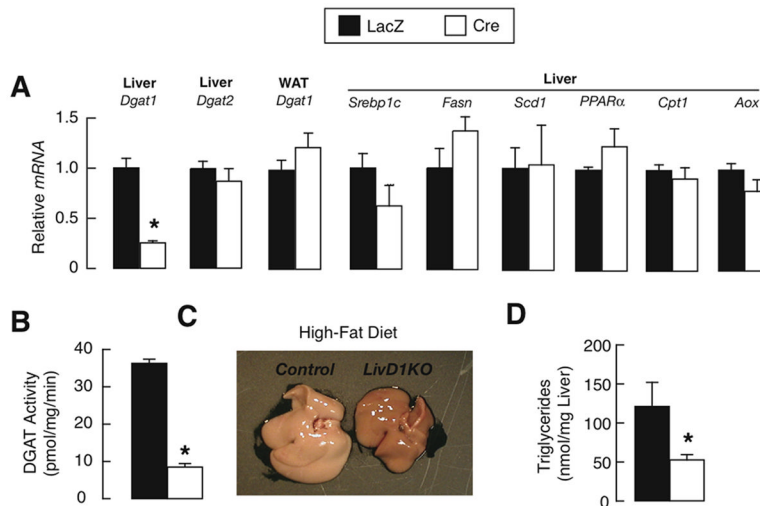
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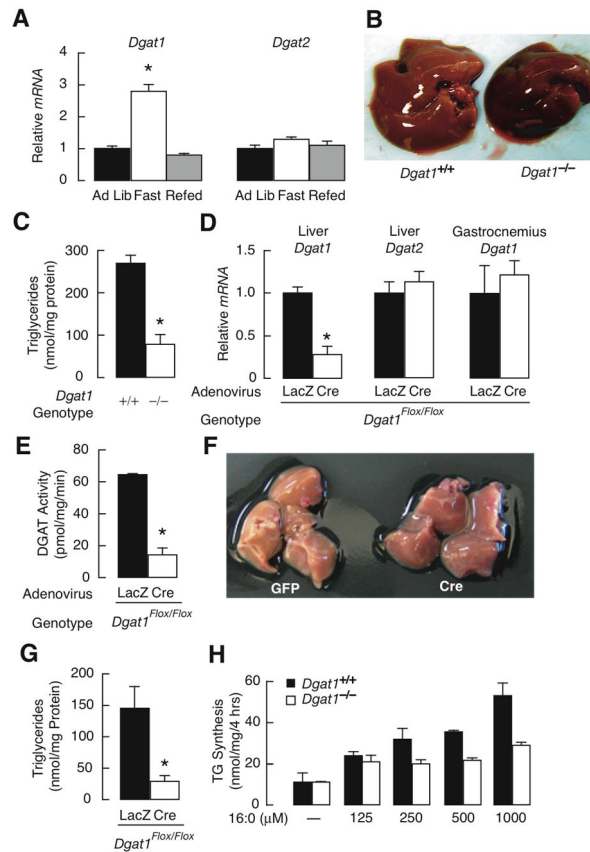
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**Fig. 1.** Protection against hepatic steatosis in *Dgat1*<sup>-/-</sup> mice fed a high-fat diet. (A) Livers and Oil-Red-O (ORO)-stained liver sections in 12-week-old mice fed a high-fat diet for 3 weeks. (B) Fatty acid (FA) composition of hepatic TG in mice fed a chow or high-fat diet (age 8–12 weeks, n=5/genotype). SFA, saturated FAs; MUFA, monounsaturated FAs; PUFA, polyunsaturated FAs. *P* \* <0.001 vs. control. (C) FA synthesis in hepatocytes from mice fed chow or high-fat diet. *P* \* <0.05 vs. chow-fed mice. \* *P*<0.001 vs. high-fat control. (D) mRNA levels of sterol regulatory element-binding protein 1c (*Srebp1c*), FA synthase (*fasn*), stearoyl-CoA desaturase 1 (*scd1*), carbohydrate response element binding protein (*Chrebp*), and liver-pyruvate kinase (*L-pk*) in livers of mice fed chow or high-fat diet. Genes were normalized to cyclophilin or 36B4 (age 8–12 weeks, n=5). \**P*<0.05 vs. control. (E) Rate of FA oxidation in hepatocytes from *Dgat1*<sup>+/+</sup> or *Dgat1*<sup>-/-</sup> mice fed chow or high-fat diet (age 12–16 weeks, n=2–3/genotype). *P* \* <0.001 vs. control. (F) mRNA levels of peroxisome proliferators-activated receptor  $\alpha$  (*Ppara*), carnitine palmitoyl-transferase 1 (*Cpt1*), and acyl-CoA oxidase (*Aox*) in livers from fat-fed *Dgat1*<sup>+/+</sup> or *Dgat1*<sup>-/-</sup> mice (age 8–12 weeks, n=5).

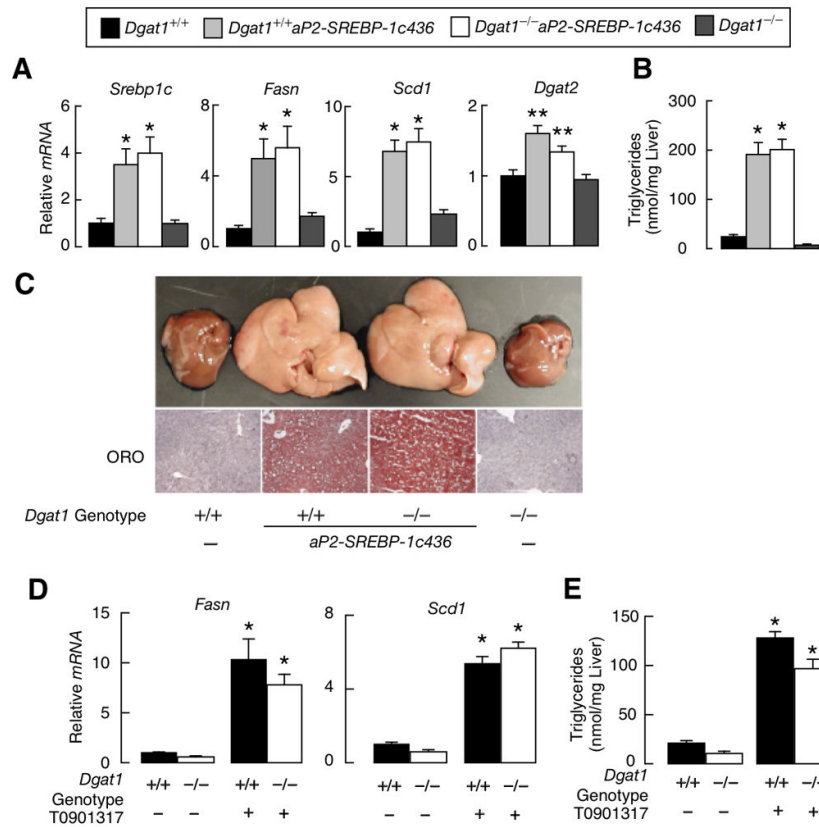


**Fig. 2.** Hepatic DGAT1 deficiency protects against hepatic steatosis induced by high-fat diet. (A) RT-PCR analysis of *Dgat1* and *Dgat2* mRNA in liver and epididymal white adipose tissue (WAT). Male *Dgat1<sup>fllox/fllox</sup>* mice received adenoviruses expressing *LacZ* or *Cre* recombinase. After 4 weeks, mice were fed a high-fat diet for 3 weeks (age 16–20 weeks, n=5–6/genotype). \*  $P < 0.001$  vs. control. (B) Microsomal hepatic DGAT activity as measured by the incorporation of [ $^{14}$ C]-oleoyl-CoA into triglycerides (age 16–20 weeks, n=5–6/genotype).  $P * < 0.001$  vs. control. (C and D) Livers (C) and hepatic triglyceride content (D) in mice fed high-fat diet (age 16–20 weeks, n=5–6 per genotype).  $P * < 0.05$  vs. control.

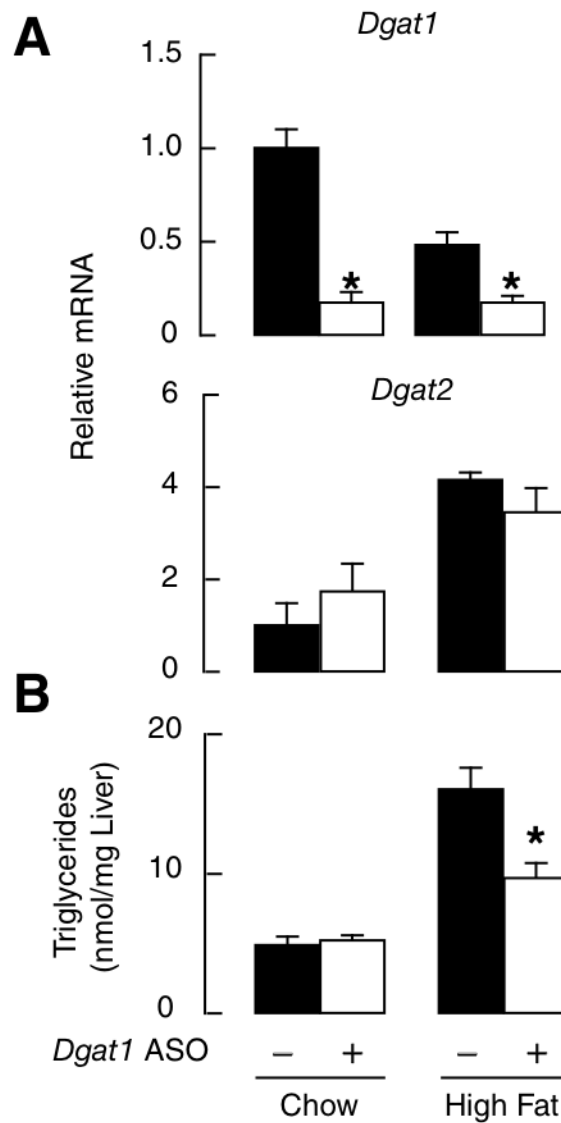


**Fig. 3.** Protection against fasting-induced hepatic steatosis in DGAT1-deficient and Liv-D1KO mice. (A) Real-time PCR analysis of *Dgat1* and *Dgat2* mRNA levels in livers of male mice fed *ad libitum* (Ad Lib), fasted for 16 h (Fast), or fasted for 24 h and refed for 12 h (Refed) (age 13–14 weeks, n=6–8/group). \**P*<0.001 vs. control. (B and C) Gross appearance of livers (B), and hepatic triglyceride content (C) after 20 hours of fasting (age 12 weeks, n=5/genotype). \**P*<0.001 vs. control. (D) Real-time PCR analysis of *Dgat1*, *Dgat2*, peroxisome proliferator-activated receptor alpha (*Ppara*), and target genes, carnitine-palmitoyl transferase 1 (*Cpt1*), aldehyde dehydrogenase 3 family member A2 (*Aldh3a2*), enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase (*Ehhadh*), and cytochrome P450, family 4, subfamily a, polypeptide 10 (*Cypa10*) mRNA levels in liver and gastrocnemius of 20 hour fasted mice. (age 16–20 weeks, n=4–5/genotype). \**P*<0.002 vs. control. (E) Microsomal hepatic DGAT activity measured by incorporation of [<sup>14</sup>C]-oleoyl-CoA into triglycerides (age 16–20 weeks, n=4/genotype). \**P*<0.001 vs. control. (F and G) Livers (F) and hepatic triglyceride content (G) in mice fasted for 20 h. (age 16–20 weeks, n=5/group). \**P*<0.01 vs. control. The experiment was repeated with similar results. (H) Triglyceride synthesis in hepatocytes challenged with palmitate (16:0) conjugated to bovine serum albumin as measured by incorporation of [<sup>14</sup>C]-Glycerol into triglycerides. The experiment was repeated with similar results.



**Fig. 4.**

DGAT1 deficiency does not protect against hepatic steatosis due to lipodystrophy or liver receptor X (LXR) activation. (A) Real-time PCR analysis of sterol regulatory element-binding protein (*Srebp1c*), fatty acid (FA) synthase (*fasn*), and stearoyl-CoA desaturase 1 (*scd1*) and *Dgat2* mRNA levels in livers of male mice (age 24 weeks, n=5–7/genotype).  $P^* < 0.001$ ,  $**P < 0.05$  vs. *Dgat1*<sup>+/+</sup>. (B) Hepatic triglyceride content in chow-fed male mice (age 24 weeks, n=7/genotype).  $P^* < 0.001$  vs. *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> mice. (C) Livers and Oil-Oed-O (ORO)-stained liver sections in 24-week-old male mice. (D) Real-time PCR analysis of FA synthase (*Fasn*) and stearoyl-CoA desaturase 1 (*Scd1*) mRNA levels in livers of mice treated with LXR agonist (T0901317, 50 mg/kg; n=5).  $P^* < 0.001$  vs. *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> mice. (E) Hepatic triglyceride content in male mice treated with 50-mg/kg T0901317 (n=5).  $P^* < 0.001$  vs. *Dgat1*<sup>+/+</sup> and *Dgat1*<sup>-/-</sup> mice.



**Fig. 5.** Pharmacologic inhibition of *Dgat1* mRNA prevents hepatic steatosis in mice fed a high-fat diet. (A) Real-time PCR analysis of *Dgat1* and *Dgat2* mRNA after treatment with control or *Dgat1* anisense oligonucleotides (ASO), administered twice weekly (50 mg/kg intraperitoneally). \* $P < 0.01$  vs. control. (B) Hepatic triglyceride content after 1 week of high-fat diet after pretreatment with control or *Dgat1* ASO ( $n=5$ ). \* $P < 0.05$  vs. control.