The triglyceride synthesis enzymes DGAT1 and DGAT2 have distinct and overlapping functions in adipocytes

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Abstract

Mammals store metabolic energy as triacylglycerols (TG) in adipose tissue. TG synthesis is catalyzed by the evolutionarily unrelated acyl CoA:diacylglycerol acyltransferase (DGAT) enzymes, DGAT1 and DGAT2, which catalyze the same reaction and account for nearly all TG synthesis. The reasons for their convergent evolution to synthesize TG remain unclear. Mice lacking DGAT1 are viable with reduced fat stores of TG, whereas DGAT2 knockout mice die postnatally just after birth with >90% reduction of TGs, suggesting that DGAT2 is the predominant enzyme for TG storage. To better understand the functional differences between the DGATs, we studied mice fed chow or high-fat diets lacking either enzyme in adipose tissue. Unexpectedly, mice lacking DGAT2 in adipocytes have normal TG storage and glucose metabolism on regular or high-fat diets, indicating DGAT2 is not essential for fat storage. In contrast, mice lacking DGAT1 in adipocytes have normal TG storage on a chow diet but moderately decreased body fat accompanied by glucose intolerance when challenged with a high-fat diet. The latter changes were associated with activation of ER stress pathways. We conclude that DGAT1 and DGAT2 can largely compensate for each other for TG storage but that DGAT1 uniquely has an important role in protecting the ER from the lipotoxic effects of high-fat diets.

Keywords

triglycerides □ adipose tissue □ high-fat diet–induced obesity □ glucose intolerance □ ER stress
Introduction

Highly reduced carbon molecules, such as triacylglycerols (TGs), are the major form of metabolic energy stores in eukaryotic cells and organisms. TGs phase separate within cells to form lipid droplets (LDs) (1-3) that exclude water and, as a consequence, provide energy-dense depots of reduced carbons. LDs can be formed in many different cell types, but for most vertebrates, the majority of TG stores are found in LDs of adipocytes. In mammals, adipocytes are found in different adipose tissues throughout the body and include subcutaneous or visceral collections of white or brown adipocytes. White adipocytes are particularly well adapted to store TGs inasmuch as they predominantly have a “unilocular” LD, which occupies the majority of the cell volume (4, 5).

The mechanisms that determine the capacity of adipocytes to store TGs are not well understood. In modern societies, humans are often confronted with an oversupply of metabolic energy, leading to excessive TG storage in adipocytes and obesity, which has become a world-wide epidemic (6). In obesity, the capacity for TG storage in adipocyte can be overwhelmed, leading to adipocyte and adipose tissue dysfunction, and by the accumulation of excess lipids in non-adipose tissues, resulting in tissue dysfunction known as lipotoxicity (7, 8). These pathological processes are thought to underlie and contribute to metabolic consequences of obesity, such as hepatic steatosis and type 2 diabetes (9-11).

In eukaryotes, TG synthesis is catalyzed by acyl CoA:diacylglycerol acyltransferase (DGAT) enzymes, DGAT1 or DGAT2 (12-14). Both catalyze the same reaction, condensing diacylglycerol and fatty acyl CoA to form TG, but they are evolutionarily unrelated (15, 16). DGAT1 and DGAT2 account for the vast majority of TG synthesis in cultured adipocytes (17, 18). A longstanding question is how the two fundamentally different enzymes contribute to TG storage in adipose tissue. DGAT1 is a member of the membrane-bound O-acyltransferase gene family (15) and is a multitopic ER membrane protein, with its likely active site on the luminal side of the ER membrane (19-23). It can transfer a fatty acyl moiety to multiple acceptors besides diacylglycerol, including retinol or long-chain alcohols to form retinyl esters or waxes, respectively (15, 24). In contrast, DGAT2 activity is largely specific to the synthesis of TG, although acylceramide synthesis activity has been reported (25) The DGAT2 protein differs from DGAT1 as it has a single, membrane-embedded hairpin with its catalytic domain on the cytoplasmatic side of the ER-membrane (26). DGAT2 additionally can re-localize around LDs where it appears to be required for LD-localized TG synthesis (26-28). Since
DGAT2 has an apparently lower $K_m$ for substrates than DGAT1 (13), it has been suggested to esterify substrates at lower concentrations for storage of TG, whereas DGAT1 may only operate at higher substrate concentrations (13).

Knockout mice deficient for either DGAT enzyme have revealed striking differences in the physiological functions of the enzymes. Mice lacking DGAT1 are viable and metabolically healthy with ~50% decreased TG stores in adipose tissue (29). These animals have increased energy expenditure, are resistant to diet-induced obesity and glucose intolerance (30, 31), and live ~25% longer (32). This complex phenotype likely involves cross-talk among different organs, including the intestine, the liver and adipose tissue (15, 29). By analyzing mice lacking DGAT1 specifically in adipocytes, we found recently that DGAT1-mediated TG synthesis not only functions in energy storage but has an additional role in protecting adipocytes from lipotoxicity during lipolysis (e.g., when fatty acids reach high intracellular concentrations as TG is rapidly hydrolyzed) (18).

In marked contrast to DGAT1 knockout mice, mice lacking DGAT2 die shortly after birth, presumably due to impaired skin permeability with increased transepidermal water loss, which leads to rapid dehydration and death (33). Importantly, DGAT2 knockout mice have dramatically reduced TG storage (with less than 10% of the normal levels), which led to the hypothesis that DGAT2 is primarily responsible for mammalian TG storage (15, 33). However, due to the vastly different phenotypes of the global knockout models, including one perinatally lethal model, this hypothesis has not been tested rigorously.

To address the question of the functional roles of DGAT1 versus DGAT2 in TG metabolism, we now generated mice lacking DGAT2 specifically in adipose tissue and compared TG synthesis and storage at basal and high-fat feeding conditions in mice lacking either DGAT1 or DGAT2 in adipose tissue (designated ADGAT1 or ADGAT2 KO mice). Unexpectedly, we find that ADGAT2 KO mice have normal TG depots and that the two enzymes can largely compensate for TG storage under basal conditions. However, important differences emerge when the mice are fed a high-fat diet. In this case, ADGAT1 KO mice store TG less efficiently and exhibit signs of lipotoxicity, shedding light on functional differences between the two enzymes.
Materials and Methods

Targeting Vector and ES Cells. Targeted ES cells were obtained from European Conditional Mouse Mutagenesis Program (EUCOMM). The targeting vector contains the L1L2_Bact_P cassette, which is composed of an FRT site (after 5′ arm which contains the *Dgat2* exon 2), followed by lacZ sequence and a loxP site. This first loxP site is followed by neomycin under the control of the human beta-actin promoter, SV40 polyA, a second FRT site and a second loxP site (34) (Fig. 1A, supplemental Fig. S1B) (before *Dgat2* exon 3). A third loxP site is inserted downstream of the targeted exons (*Dgat2* exon 4). The critical exons (exon 3 and 4) are thus flanked by loxP sites. A "conditional ready" (floxed) allele can be created by flp recombinase expression in mice carrying this allele. Subsequent cre expression results in a knockout mouse. If cre expression occurs without flp expression, a reporter knockout mouse will be created. The vector was electroporated into JM8A1.N3 ES cell line from C57BL/6N mice (35).

ES Cell Culture, Blastocyst Injections, and Transfer to Foster Mothers. We obtained two different ES cell clones from EUCOMM. ES cells were thawed and then cultured on mitomycin C–treated feeder cells (STO feeder cell line), as per the protocols provided by EUCOMM. ES cells culture medium contained, 500 ml of knockout DMEM (GIBCO, cat # 10829), 90 ml of FBS (GIBCO, lot tested), 5 ml of 100x L-glutamine (GIBCO, cat # 25030), 5 μl of 100x β-mercaptoethanol (Sigma, cat # M7522; 360 μl/500 ml PBS (100x), sterile filtered, stored at -20ºC), 100 μg/ml G418 (Gibco/Invitrogen, cat # 10131-027), ESGRO (LIF; Chemicon, cat # ESG1107). Total DNA from ES cells was isolated and tested by PCR for neomycin phosphotransferase II to confirm the presence of the targeted vector. For blastocyst injections, ES cells were trypsinized when they were at 50–70% confluency (supplemental Fig. S1A) (as per protocol provided by EUCOMM) and submitted to transgenic core facility, Gladstone Institutes, San Francisco, CA, for blastocyst injections and transfer to foster-mothers.

Generation of DGAT2 KO and DGAT2 flox, ADGAT2 KO and SDGAT2 KO Mice. Founders (chimeras) were identified by PCR for neomycin phosphotransferase II (supplemental Fig. S1A). Transmission of mutant allele was confirmed by crossing founders with C57BL/6J mice. DGAT2 global KO (DGAT2 KO) mice were generated by crossing the mice heterozygous for gene-trap allele. To generate *Dgat2* ^\text{flox/flox}\text{ (DGAT2 flox)} mice, heterozygous mice for gene-trap allele were crossed with transgenic mice expressing FLP-e recombinase under the control of human beta-
actin promoter (supplemental Fig. S1D) (36). Deletion of gene-trap cassette and orientation of \( \text{lox-p} \) sites were confirmed by PCR. ADGAT2 KO mice were generated by crossing DGAT2 flox mice with transgenic mice expressing CRE recombinase under control of mouse adiponectin promoter (37). We generated SkiDGAT2 KO mice by crossing DGAT2 flox mice with transgenic mice expressing CRE recombinase under control of the human keratin-14 promoter (38).

**Mouse Husbandry and Dietary Intervention Studies** All animal experiments were performed under the guidelines established by Harvard Center for Comparative Medicine (HCCM). Mice were maintained in a barrier facility, at normal room temperatures, on a regular 12-h light and 12-h dark cycle and had *ad libitum* access to food and water unless otherwise stated. Mice were fed on standard laboratory chow diet (PicoLab Rodent Diet 20 5053) or Western-type high-fat diet (Envigo, TD.88137).

**Mouse DGAT1 and DGAT2 Antibody Generation.** We custom generated rabbit polyclonal antibodies against mouse DGAT1 and mouse DGAT2 (GenScript, Piscataway, NJ). To generate mouse DGAT1 antibodies, we used an N-terminal peptide corresponding to amino acids 23–52 (NH2-GGSGPKVEEDEVR DAAVSPDLGAGGDAPAP-COOH) of mouse DGAT1 as the antigen. The peptide was conjugated to mcKLH with the Imject EDC mcKLH spin kit (Thermo Scientific, cat. # 77671), according to the manual. The antigen was then injected into rabbits to produce the antibody. We injected five rabbits, and two gave better immune response. To generate mouse DGAT2 antibodies, we used a C-terminal peptide corresponding to amino acids 373–388 (NH2-KTKFGLPETEVLEVN-COOH) of mouse DGAT2 as the antigen. We injected five rabbits, and two gave an appropriate antibody. Antibodies were affinity purified, and used at a 1:1,000 dilution in 5% milk in TBST, incubate over night at 4°C.

**DGAT Activity Assay.** DGAT enzymatic activities were measured in membrane fractions isolated from WAT of wild-type and DGAT1 KO mice, respectively. Enzymatic activities were measured at Vmax substrate concentrations. Assay mixture contained 5–10 µg of membrane proteins, 100 µM of 1,2-dioleoyl-sn-glycerol, 25 µM of oleoyl-CoA, which contained [14C] oleoyl-CoA as tracer, 25 mM MgCl\(_2\) for the DGAT1 assay and 1 mM MgCl\(_2\) for the DGAT2 assay in an assay in buffer containing 100 mM Tris-HCl (pH 7.4) and protease inhibitors. Utilizing 25 mM MgCl\(_2\) or greater in
the assay renders it largely specific for DGAT1 in adipocytes (13, 18). Reaction was carried out as described earlier (18). After stopping the reaction, lipids were extracted, then separated by TLC using hexane:diethyl ether:acetic acid (80:20:1) solvent system. The TLC plates were exposed to phosphor imager screen and developed. TLC plate were exposed to phosphor imaging cassette overnight and revealed by Typhoon FLA 7000 phosphor imager.

**RNA Extraction and Quantitative Real-Time PCR (qRT-PCR).** Total RNA was isolated using the RNeasy Kit (Qiagen), according to the manufacturer’s instructions. For isolating RNA from WAT, an RNeasy Lipid Tissue (Qiagen) was used. Complementary DNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad), and qPCR was performed in triplicates using SYBR Green PCR Master Mix Kit (Applied Biosystems). (Sequences of the qPCR primers used are listed in Table S1).

**Tissue Lipid Analysis.** Liver and skeletal muscle were homogenized in 1 ml of lysis buffer (0.25 M sucrose, 50 mM Tris HCl, pH 7.0 with protease inhibitor cocktail) using a Misonix Sonicator 4000. The homogenate was mixed with 5 ml of chloroform:methanol (3:2 v/v) and extracted overnight by rotation. Upon centrifugation at 3000 x g at room temperature for 10 min, 100 µl of lower organic phase was collected and dried in a speed vac. To the dried lipids, 100–300 µl of 0.1 % Triton X-100 was added and sonicated. TG and total cholesterol were measured using Infinity TM Triglycerides reagent (Thermo Scientific) and a cholesterol E kit (Wako Diagnostics), respectively, according to manufacturer’s protocol. For plasma lipids measurement, 5 µl of plasma was used directly.

**Immunoblotting.** Cells were lysed using RIPA lysis buffer. Tissues were lysed in buffer containing 250 mM sucrose, 100 mM Tris-HCl (pH 7.4), and protease inhibitors in a Dounce homogenizer. Proteins were denatured in Laemmli buffer, separated on 10% SDS-PAGE gel, and transferred to a PVDF membrane (Bio-Rad). Membranes were blocked with blocking buffer for 2 h in TBST containing 5% BSA or 5% milk, and then incubated with primary antibodies overnight. The membranes were then washed with TBST for 10 min x 3 times, and incubated in mouse secondary antibodies (Santa Cruz Biotechnology) at 1:5000 dilutions in blocking buffer. Membranes was washed again with TBST for 10 min x 3 times and revealed using the Super Signal West Pico kit (Thermo Scientific). DGAT1 antibodies were a
gift from Dr. Jin Ye from UT Southwestern Medical Center. CHOP and Bip antibodies were purchased from Cell Signaling Technology.

**Statistical Analyses.** Data are presented as mean± SD (standard deviation) or mean± SEM (standard error mean). Statistical significance was evaluated by unpaired two-tailed Student’s t-test or two-way ANOVA with repeated measures.
Results

Mice Lacking DGAT2 in the Epidermis Die Postnatally, but Mice Lacking DGAT2 in Adipose Tissue Are Viable.

To analyze the contribution of DGAT2 to TG synthesis in different cell types, we generated tissue-specific knockouts of DGAT2 by using a gene-trap allele that allows for the generation of either whole body or tissue-specific knockout mice (34) (Fig. 1A). We first generated DGAT2 global knockout mice by intercrossing mice that were heterozygous for the targeted gene-trap allele (Fig. 1A). Consistent with our previous findings (33), DGAT2 KO mice generated by this allele died shortly after birth and had an apparent defect in the epidermal water-permeability barrier (Fig. 1B, 1C), including increased uptake of toluidine blue (a water-based dye). These findings validate that the gene-trap allele correctly targeted and inactivated the Dgat2 gene.

Previous studies did not resolve whether the skin abnormalities of DGAT2 knockout mice resulted from loss of DGAT2 function in the skin or other tissues (33). We therefore generated skin-specific DGAT2 knockout mice (SkiDGAT2 KO) by crossing Dgat2flox/flox (DGAT2 floxed) mice with transgenic mice expressing Cre recombinase under control of the human keratin-14 promoter (38) (Fig. 1A). SkiDGAT2 KO mice were markedly abnormal in appearance and died shortly after birth, essentially phenocopying the skin permeability-barrier defect of DGAT2 KO mice (Fig. 1D, 1E). Western blot analyses confirmed that DGAT2 was absent in the epidermis of SkiDGAT2 KO mice (Fig. 1F). These findings indicate that DGAT2 has an essential and tissue-autonomous function to maintain skin lipids important for the epidermal water barrier in mice.

We next generated ADGAT2 KO mice by crossing DGAT2 floxed mice with transgenic mice expressing Cre recombinase under the control of the murine adiponectin promoter (Fig. 1A) (37). In contrast to the SkiDGAT2 KO mice, ADGAT2 KO mice were viable and appeared healthy, with no apparent defects in their skin. Real time-qPCR analysis showed Dgat2 mRNA levels were decreased by ~85% in gonadal white adipose tissue (gWAT) and in brown adipose tissue (BAT) of ADGAT2 KO mice, but were unchanged in livers and in skeletal muscle (Fig. 1G). We found no evidence for compensatory up-regulation of DGAT1 mRNA expression in gWAT and BAT of ADGAT2 KO mice (Fig. 1G). Western blot analyses showed complete lack of DGAT2 protein in gWAT of ADGAT2 KO mice, confirming complete KO of DGAT2 in adipose tissue (Fig. 1H).
DGAT1 and DGAT2 Are Functionally Redundant for TG Storage in Adipocytes under Basal Conditions. Based on the dramatic reduction of TG levels in animals lacking DGAT2 globally (33), we expected that ADGAT2 KO mice would have markedly reduced TG levels in adipose tissue, possibly even resulting in lipodystrophy. Unexpectedly, we found that ADGAT2 KO mice fed a chow diet had normal body weight and fat depots (Figs. 2A, 2B). Moreover, plasma levels of glucose, insulin, non-esterified fatty acids, and TG were similar in ADGAT2 KO mice and DGAT2 flox (control) mice fed a chow diet (Fig. 2C). Additionally, WAT, BAT, and liver all had normal histological appearances in ADGAT2 KO mice (Fig. 2D). Thus, unexpectedly, ADGAT2 KO mice had normal TG storage with chow feeding. Under these conditions, ADGAT1 KO mice had a virtually identical phenotype to ADGAT2 KO mice, with the exception of a modest 20% reduction in gonadal fat weight (Fig. 2A, 2D).

Reduced TG Storage in Fat and Impaired Glucose Tolerance in ADGAT1 KO Mice fed a High-Fat Diet.

To determine the relative contributions of each DGAT enzyme to TG storage during excess fat intake, we fed ADGAT1 KO mice, ADGAT2 KO mice, or control mice a Western-type high-fat diet (HF diet) for 12 (Fig. 3A) or 20 weeks (supplemental Fig. S2A, S2B). During this time, ADGAT1 KO mice gained ~10% less body weight than ADGAT2 KO mice or either floxed (control) line of mice, and this reduction in body weight was due to a ~30% reduction in fat mass, with 40% and 30% reductions in gonadal or brown fat masses, respectively (Fig. 3B, 3C). Levels of liver and skeletal muscle TGs were moderately higher in ADGAT1 KO mice fed a high-fat diet than controls (Fig. 3D). With respect to plasma metabolites, ADGAT1 KO mice showed ~10% increases in plasma levels of glucose, insulin, and free fatty acid levels (Fig. 3E), and impaired glucose and insulin tolerance (Fig. 3H). In contrast, we found no differences in body weight, fat mass, plasma parameters, or glucose or insulin tolerance in ADGAT2 KO (Fig. 3A, 3E). Under these conditions, DGAT1 protein levels in ADGAT2 KO mice were not upregulated (Fig. 3F), indicating a lack of compensation in expression, although the capacity for TG synthesis activity for DGAT1 was increased by ~25%, suggesting post-translational regulation (Fig. 3G). For ADGAT1 KO mice, there were no compensatory changes in DGAT2 protein levels.

High-Fat-Diet Feeding Causes Differential Gene Expression Changes in Mice Lacking DGAT1 or DGAT2 in Adipose Tissue. Our findings are consistent with high-fat feeding provoking a lipotoxic response when DGAT1 is
lacking in adipocytes. We therefore examined the expression of ER stress and inflammatory marker genes in gWAT of ADGAT1 KO mice fed the high-fat diet. We found greater expression of target genes of the unfolded protein response, most notably for the PERK branch of this signaling pathway (Atf4 and Chop), in ADGAT1 KO mice than controls (Fig. 4A). Consistent with previous findings that established correlations between UPR and inflammatory gene expression (39, 40), we found increased expression of cytokines Mcp1 and Tnfa in gWAT of high-fat fed ADGAT1 KO mice (Fig. 4A). We also found modest increases in protein levels of ER stress markers BIP and CHOP in gWAT of high-fat fed ADGAT1 KO mice (Fig. 4C). Thus, ADGAT1 KO mice fed a high-fat diet exhibit gene expression findings consistent with an ongoing lipotoxic response.

In contrast, ADGAT2 KO mice fed the high-fat diet did not exhibit gene expression changes of lipotoxicity (Fig. 4B), likely because DGAT1 is expressed. Because we and others showed previously that DGAT2 mRNA levels exhibit a positive correlation with the expression of de novo lipogenic genes of the SREBP1 pathway (41, 42), we examined this in adipose tissue of ADGAT2 KO mice. Consistent with previous reports, we found reduced levels of mRNA for Srebp1c and numerous Srebp1c target genes (Fig. 4B). Such changes were not found in ADGAT1 KO fat, with the exception of a reduction of mRNA levels of Scd1 (Fig. 4A). Under conditions of a high-fat dietary challenge, DGAT1 or DGAT2 inactivation resulted in very different effects with respect to gene expression, with DGAT1 expression being inversely correlated with ER stress and DGAT2 expression being positively correlated with lipogenesis.
Discussion

By studying murine models lacking either DGAT1 or DGAT2 in adipocytes we can draw several important conclusions with respect to the functions of the two enzymes in TG and energy metabolism. First, and most surprisingly given the previous model that DGAT2 might be the major enzyme responsible for TG synthesis and storage in mice (15, 33), we now find that the two enzymes are functionally redundant with respect to TG storage in animals fed a chow diet. During basal conditions of chow feeding, deletion of either enzyme in adipocytes resulted in little to no detectable metabolic phenotype, showing that the enzymes can compensate for each other. This finding is perhaps not totally unexpected, given that treatment of many different cell types with inhibitors of either DGAT enzyme does not result in marked reduction of TG synthesis (17, 18). Additionally, these findings are consistent with in vitro analyses of DGAT1 and DGAT2 in mouse embryonic fibroblast and in 3T3-L1-derived adipocytes where TG synthesis activity from either enzyme was sufficient to support TG accumulation during differentiation (17, 18). It appears that either DGAT enzyme is fully capable of catalyzing TG synthesis for storage under basal conditions or during adipocyte differentiation.

A second major conclusion was evident when we challenged mice with a high-fat diet. Specifically, only ADGAT1 KO mice were protected (to some degree) from diet-induced obesity. Biochemically, this could be due to preferential channeling of exogenously provided fatty acids to DGAT1 at relatively high substrate concentrations (13, 29, 43). Presumably, DGAT2 is unable to compensate for DGAT1 when high levels of fatty acids are delivered exogenously to cells. For many models, increased leanness is associated with improved glucose and energy metabolism. However, the protection from TG accumulation in ADGAT1 KO mice fed a high-fat diet was accompanied by metabolically detrimental effects consistent with lipotoxicity, including activated ER stress, inflammation, and apparent insulin resistance. That DGAT1-mediated TG synthesis is protective against fatty-acid-induced lipotoxicity is becoming abundantly clear. DGAT1 overexpression in several different transgenic mouse models protects these mice from the toxic effects of lipids (41, 43-46). Also, selective deletion of DGAT1 in several tissues in mice predisposes these mice to lipotoxicity (47, 48), and humans who lack DGAT1 exhibit dietary fat–induced diarrhea (49). We also found that DGAT1 mRNA levels in human adipose tissue exhibits and inverse correlation with many genes of ER stress (18). All of these studies are consistent with the hypothesis that DGAT1-mediated TG synthesis is lipo-protective for cells and tissues.
Of note, whole-body deletion of DGAT1 in mice paradoxically results in a metabolically protective phenotype rather than one of lipotoxicity (29-31). Two factors likely contribute to this. First, mice express DGAT2 in their small intestine (humans do not) (50), so they are presumably mostly spared from the lipotoxic effects dietary fat could cause in the intestine when DGAT1 is deleted. Second, DGAT1 knockout mice exhibit a complex phenotype with activation of energy expenditure (29, 30), including increased fatty acid oxidation (51), that likely prevents the accumulation of lipids in tissues. This phenotype includes complex metabolic effects resulting in improved insulin and leptin sensitivity (30) for reasons that are still poorly understood but may result from changes in secreted factors (52).

Previously, we showed that DGAT2 is essential for mice (33). We now provide evidence, through an epidermis-specific deletion, that one essential function for the enzyme is a cell type-autonomous, crucial role in maintaining the transepidermal water barrier. How DGAT2 contributes to the barrier is currently unclear. One possibility is that the enzyme is required for the production of specific ω-0-acylceramides levels that are thought to be crucial for the lipids of the corneocyte envelope involved in forming the water barrier (53) and which are reduced in the skin of DGAT2 KO mice (33). In addition to DGAT2, loss of other lipid metabolism genes in murine skin also result in a similar phenotype (53-55), indicating a complex and crucial pathway of lipid metabolism in this cell type. With respect to DGAT2, the enzyme may either directly contribute to the synthesis of these lipids, or function indirectly by enabling storage of precursors for their synthesis. Humans with DGAT2 deficiency appear not to have such marked skin abnormalities, although DGAT2 function has been linked to psoriasis (56).

Finally, our study sheds additional light on DGAT2 function. The current study shows that DGAT2 can function to esterify fatty acids generally if concentrations are not too high, and there is an emerging connection of DGAT2 with de novo lipogenesis. This includes several studies that have linked DGAT2 activity to de novo synthesis of fatty acids (43, 57), and this and other studies (42, 58) that suggest a feedback mechanism correlating DGAT2 activity with the SREBP1-mediated pathway. Notably DGAT2 is evolutionarily conserved as a major TG synthesis enzyme across eukaryotes (15, 16), whereas DGAT1 is a member of the MBOAT gene family, several of whose lipid-esterifying members have been linked to the detoxification of lipids in the ER (59). Thus, collectively, a picture emerges in which DGAT2 has a more ancient function for mediating TG synthesis of de novo–synthesized fatty acids, and DGAT1, which can utilize a variety of acyl acceptor substrates (15, 24), has a specific function in ER protection.
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Fig. 1. Dgat2 whole body knockout mice (DGAT2 KO) and skin-specific Dgat2 knockout mice (SkiDGAT2 KO) die shortly after birth.

(A) Strategy for generation of DGAT2 KO, Dgat2(DGAT2 flox) mice, and tissue specific Dgat2 knockout mice. A 'knock-out first/conditional-ready' gene-targeting vector was used to generate targeted ES cells. A gene-trap cassette is located between two FRT sites. LacZ, b-galactosidase; neo, neomycin-phosphotransferase II. (B and C) DGAT2 KO mice die within few hours after birth due to defective skin barrier function. (D and E) SkiDGAT2 KO mice were generated by crossing DGAT2 flox mice with the mice expressing CRE-recombinase under control of the human keratin-14 promoter. SkiDGAT2 KO mice die within few hours after birth due to defective skin barrier function. (F) DGAT2 is absent in epidermis of SkiDGAT2 KO mice (n=3 mice). (G) Adipose tissue-specific Dgat2 knockout mice (ADGAT2 KO) were generated by crossing DGAT2 flox mice with the mice expressing CRE-recombinase under control of the mouse adiponectin promoter. Dgat1 and Dgat2 mRNA levels in gWAT of DGAT2 flox and ADGAT2 KO mice (n=5 mice). (H) DGAT2 is absent in gWAT of ADGAT2 KO mice (n=4 mice). Data are presented as mean ± SD. ***p<0.001.
2. DGAT1 and DGAT2 largely compensate for TG storage in adipose tissue in chow fed state.

Body weights of chow diet fed mice (n=10 to 12 mice). (B) Organ weights of chow diet fed mice (n=8 mice per genotype). (C) Plasma parameters of chow diet fed mice (n=7 mice per genotype). (D) H&E stained sections of gWAT, iBAT, and livers of chow diet fed mice (representative images of n=8 mice per genotype). Scale bars, 50 µm. Data are presented as mean ± SD. *p<0.05, by t-test.
Fig. 3. DGAT1 contributes more to diet induced obesity. (A) Body weights of high fat-diet (HFD)-fed mice (n=15). (B) Lean mass and fat mass analysis of HFD fed mice (n=10 mice per genotype). (C) Weights of gWAT and iBAT of HFD-fed mice (n=8 mice). (D) TG content of livers and sk. muscle (n=6 mice). (E) Plasma parameters of HFD fed mice (n=6 mice). (F) Western blot analysis of tissues from HFD fed mice (n=4 mice). (H) DGAT1 activity in gWAT (n=4 mice). (G) Glucose-tolerance and an insulin-tolerance tests were performed on HFD-fed mice (n=10 to 16 mice). Data are presented as mean ± SD (C, D, and E), as mean ± SEM (A, B, G, and H). *p<0.05, **p<0.01 by t-test; ***p<0.01 by two-way ANOVA.
4. ER stress in HFD-fed ADGAT1 KO mice, but not in ADGAT2 KO mice. (A and B) mRNA levels of lipogenic, ER stress genes, and inflammatory genes determined by RT-qPCR. (n=6 mice per genotype). Mice were fed HFD for 16 weeks. Tissues were collected in ad libitum fed state (without food for 2 h before collecting tissues). Protein levels of BIP and CHOP in gWAT of 16 weeks HFD fed mice (n=4 mice). Data are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001 by t-test.