Cloning of DGAT2, a Second Mammalian Diacylglycerol Acyltransferase, and Related Family Members*

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Studies involving the cloning and disruption of the gene for acyl-CoA:diacylglycerol acyltransferase (DGAT) have shown that alternative mechanisms exist for triglyceride synthesis. In this study, we cloned and characterized a second mammalian DGAT, DGAT2, which was identified by its homology to a DGAT in the fungus Mortierella ramanniana. DGAT2 is a member of a gene family that has no homology with DGAT1 and includes several mouse and human homologues that are candidates for additional DGAT genes. The expression of DGAT2 in insect cells stimulated triglyceride synthesis 6-fold in assays with cellular membranes, and DGAT2 activity was dependent on the presence of fatty acyl-CoA and diacylglycerol, indicating that this protein is a DGAT. Activity was not observed for acyl acceptors other than diacylglycerol. DGAT2 activity was inhibited by a high concentration (100 mM) of MgCl₂ in an in vitro assay, a characteristic that distinguishes DGAT2 from DGAT1. DGAT2 is expressed in many tissues with high expression levels in the liver and white adipose tissue, suggesting that it may play a significant role in mammalian triglyceride metabolism.

Triglycerides (triacylglycerols) are neutral lipids found in most eukaryotic cells, where they serve as highly reduced stores of oxidizable energy. Because of their hydrophobicity, triglycerides are concentrated in cytosolic lipid droplets in cells or are found in the plasma as core components of lipoproteins. Excess storage of triglycerides in adipocytes results in obesity, which is increasing in prevalence in humans at alarming rates (1). Understanding the mechanisms of triglyceride synthesis is of potential importance for elucidating the molecular processes that contribute to obesity and other disorders of triglyceride metabolism.

Until recently, it was believed that a single enzyme, acyl-CoA:diacylglycerol acyltransferase (DGAT, EC 2.3.1.20), was responsible for triglyceride synthesis (2–4). DGAT catalyzes a reaction in which diacylglycerol is covalently joined to long chain fatty acyl-CoAs. Diacylglycerol for this reaction comes from the glycerol-phosphate pathway, in which glycerol phosphate is sequentially acylated at the sn-1 and sn-2 positions by acyltransferases, followed by the removal of the phosphate group of phosphatidic acid to form diacylglycerol. The glycerol-phosphate pathway and DGAT activity are present in most tissues (5). In some cells, such as enterocytes (2, 6) and adipocytes (7), diacylglycerol may also be derived directly from monoacylglycerol in a reaction catalyzed by monoacylglycerol acyltransferase.

Previously, we identified a cDNA encoding a DGAT (8). Homologues for this DGAT gene have been identified in many species, including plants and humans (9–11). To investigate the in vivo function of this gene, we generated DGAT-deficient (Dgat⁻/⁻) mice (12). These mice have reduced body fat and are resistant to diet-induced obesity through a mechanism that involves increased energy expenditure (12). In addition, female Dgat⁻/⁻ mice have a lactation defect. Surprisingly, however, Dgat⁻/⁻ mice have normal plasma triglyceride levels and abundant triglycerides in their adipose tissue, indicating that alternative mechanisms exist for synthesizing triglycerides.

How are triglycerides synthesized in Dgat⁻/⁻ mice? One possibility is from the activity of another DGAT. Biochemical studies have provided evidence for more than one DGAT activity in rat liver (13, 14). One activity appears to be more closely coupled with synthesizing triglycerides for storage in the cell and the other with secretion of triglycerides for lipoprotein assembly. Studies of rat intestine have suggested that a second DGAT may exist in this tissue as part of a multienzyme complex (15). A second possibility is that triglyceride synthesis may occur by acyl-CoA-independent mechanisms, such as by the actions of transacylase enzymes. Indeed, a diacylglycerol transacylase activity, in which a fatty acyl chain is transferred from one diacylglycerol molecule to another, has been reported in rat liver and small intestine (16). In addition, a transacylase activity in which a fatty acyl chain is transferred from phosphatidylcholine to diacylglycerol appears to account for the majority of triglyceride synthesis in yeast (17, 18), although this activity has not been reported in mammals.

Recently, a DGAT enzyme without sequence homology to previously identified DGAT genes was isolated by protein purification from the fungus Mortierella ramanniana (26). In the present study, we report the identification of a mammalian gene family related to this fungal DGAT. Furthermore, we report the cloning and functional characterization of one member of this family in mammalian cells.
and human testis (hDGAT2). The complete coding sequence for mDC3 length cDNAs were amplified from mouse liver (mDGAT2 and mDC2) designed, and amplifications were performed from tissue cDNA. Full-processed through workbench.sdsc.edu, based on homology with the other genomic sequences of the high throughput sequencing data base (ac-

polymerase chain reaction. Human cDNA sequences were deduced from length cDNAs were generated by rapid amplification of cDNA ends and primers for amplifications were derived from EST sequences, and full-

mouse genes and their human homologues and for two human genes BLAST data base. Expressed sequence tags (ESTs) for three different (20). Cells were plated at a density of 3

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were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in a 7% CO₂ incubator. Pulse assays were then performed using a protocol similar to that described by Goldstein et al. (20). Cells were plated at a density of 3 × 10⁵ cells per well on six-well plates. On the following day, the medium was replaced with fresh culture medium containing 1 nm [¹⁴C]oleic acid provided as a [¹⁴C]oleic acid-bovine serum albumin (BSA) complex (10% BSA, 10 mM sodium oleate; specific activity: 2 μCi/μmol), and the cells were cultured for 24 h at 37 °C. The cells were then washed three times with phosphate-buffered saline containing 0.2% BSA and twice with phosphate-buffered saline alone, and then cellular lipids were extracted with hexane:isopropanol (3:2 v:v). Lipid extracts were separated by thin-layer chromatography (TLC), the triglyceride band was isolated, and radio-

activity was quantified by scintillation counting.

Mouse adipocytes were isolated by collagenase treatment of pooled reductive fat pads isolated from three wild-type or Dgat⁻/⁻ mice (21). The fat pads were cut into small pieces, rinsed in 5 ml of Krebs-Ringer phosphate buffer, and incubated at 37 °C for 30 min in 3 ml of collagenase solution (2% collagenase (Sigma), 1% BSA in Krebs-Ringer phosphate buffer). The resulting adipocyte suspension was washed twice in Krebs-Ringer phosphate buffer, and the volume of adipocytes was estimated and diluted in buffer to obtain a 25% (v:v) cell suspension. Aliquots (100 μl) of this cell suspension were transferred into plastic tubes in a water bath at 37 °C. The assay for triglyceride synthesis was started by adding 10 μl of [¹⁴C]oleic acid-BSA complex (0.1 μCi/μl; 2000 dpm/μl) and the cells were incubated for 24 h. Cellular lipids were extracted with 1 ml of hexane:isopropanol (3:2 v:v) and 0.5 ml of distilled water. An aliquot (10 μl or ~2000 dpm) of [³H]triolein (Amersham Pharmacia Biotech) was added to each sample before extraction to assess recovery. Lipids were separated by TLC, and the triglycerides were isolated and quantified as described above.

Insect Cell Expression Studies—The human and mouse DGAT2 cod-
ing sequences with or without an N-terminal FLAG epitope (MDDYKDDDG, epitope underlined) were subcloned into pVL1392 or pVL1393 baculovirus vectors (PharMingen). Recombinant baculoviruses were generated by cotransfecting the transfer vectors with BaculoGold DNA (PharMingen), and high viral titers were obtained from two rounds of amplification in SF9 insect cells (cultured in Grace’s medium (Life Technologies) and 10% fetal bovine serum at 27 °C) as described (8). For protein expression, Sf9 cells were infected, and the membrane fraction was isolated as described (8). Membrane samples were immunoblotted with an anti-FLAG M2 monoclonal antibody (Sigma), followed by detection with enhanced chemiluminescence (Amersham Pharmacia Biotech).

DGAT Activity Assays—DGAT activity assays were performed by measuring activity under apparent Vmax conditions in tissue mem-

branes (10–50 μg of protein) as described (6), except that exogenous diglycerol was added in acetone (6). In brief, these assays measured the incorporation of [¹⁴C]oleoyl-CoA (specific activity: ~20,000 dpm/ nmol) into triglycerides in a 5-min assay using 0.4 mM diglycerol and 25 μM oleoyl-CoA as substrates. In some assays, [¹⁴C]sn-1,2-diacyl-

glycerol (specific activity: 12.5 μCi/μmol, 0.4 mM final concentration) was used as an acyl acceptor with unlabeled oleoyl-CoA (25 μM). Different concentrations of MgCl₂ (0–100 mM) were used in specific exper-

FIG. 1. Residual DGAT activity in Dgat⁻⁻ mice. A, comparison of DGAT activity in membranes and intact cells. For measuring DGAT activity in membranes, the membrane fraction was isolated from MEF cells or adipose tissue and assayed as described under “Materials and Methods.” The MgCl₂ concentration in the assay was 100 mM. For measuring triglyceride synthesis in intact cells, monolayers of MEF cells in six-well culture dishes or freshly isolated adipocytes cultured in suspension in Krebs buffer were labeled for 24 h with [¹⁴C]oleic acid. Total lipids were extracted and separated by TLC to quantify incorporation of [¹⁴C] into triglycerides. Results are expressed as the percentage of activity in wild-type membranes or cells and are the mean ± S.D. of three experiments. Open bars represent wild-type, and filled bars are Dgat⁻⁻. B–C, effect of MgCl₂ on DGAT activity in tissue membranes. In B, membrane fractions were isolated from brain, heart, or skeletal muscle of wild-type or Dgat⁻⁻ mice and assayed for DGAT activity with or without MgCl₂ (100 mM) in the assay mix. Results are the mean ± S.D. of three experiments. *, p < 0.01 versus Dgat⁻⁻ tissue assayed with 100 mM MgCl₂. In C, membranes from liver and adipose tissue were assayed for DGAT activity using a range of MgCl₂ concentrations in the assay mix. The experiment was repeated three times with similar results. A representative experiment is shown.
**RESULTS**

Identification of a Second DGAT Activity in Dgat−/− Mice—DGAT activity, measured by our standard DGAT assay (8), is nearly absent in membranes from most tissues of Dgat−/− mice (12). However, significant amounts of triglycerides are present in these mice (12). To further examine the ability of Dgat−/− tissues to synthesize triglycerides, we compared triglyceride synthesis in isolated membranes with that in intact MEF cells and adipocytes. Although DGAT activity was nearly absent in the membrane preparations, the incorporation of radiolabeled oleic acid into triglycerides in intact MEF cells and adipocytes was 37 and 48%, respectively, of that in Dgat+/+ cells (Fig. 1A). Thus, these Dgat−/− cells were capable of synthesizing triglycerides.

We hypothesized that residual triglyceride synthesis in Dgat−/− tissues may reflect the action of a second DGAT whose activity was not detected under our standard assay conditions. To test this hypothesis, we altered the conditions of the in vitro DGAT assay. Removing MgCl₂ from the assay buffer resulted in the detection of a second DGAT activity. The activity was not observed in the presence of MgCl₂, indicating that MgCl₂ is essential for the in vitro assay.

![Image](image-url)
in increased DGAT activity in membranes from Dgat−/− liver and adipose tissue when MgCl₂ concentrations less than 100 mM were used, with maximal activity at 5 mM MgCl₂ for both tissues (Fig. 1C). The level of DGAT activity in Dgat−/− adipose tissue with 5 mM MgCl₂ in the assay (∼1000 pmol of triglyceride formed per mg of protein/min) was comparable to that normally found in mouse liver.

**Identification of a DGAT2 Gene Family**—To identify a cDNA that encoded a second DGAT activity, we performed homology searches of sequence databases using coding sequences for the first-cloned DGAT (DGAT1). However, only ACAT1 and ACAT2 homologues, which are known members of the DGAT1 gene family (23), were found. Recently, a DGAT was isolated by protein purification in the fungus M. rammaniana (26). The gene encoding this DGAT (MrDGAT2A) has no sequence homology with members of the DGAT1 family, including genes related to plant DGAT1 (9, 10). Using sequences of the newly identified fungal DGAT to perform homology searches of EST data bases, we identified a family of related genes in mammalian species (Fig. 2A). Using methods based on the polymerase chain reaction or by deducing the cDNA sequence from the gene sequence of large genomic clones, we determined the full-length cDNA sequence for three murine and five human DGAT2-related genes. In the present study, we focused on the functional characterization of one of these genes, which we have named DGAT2. We have designated the other gene family members as DGAT candidate genes until it is determined whether the encoded proteins possess DGAT activity.²

The mouse and human DGAT2 cDNAs are predicted to encode proteins of 388 and 387 amino acids, respectively. The sequence for mouse DGAT2, which is 43% identical to the M. rammaniana DGAT, is shown in Fig. 2B. Analyses of the predicted protein sequences (TMHMM program, workbench.sdsu.edu) consistently identified one potential transmembrane domain and the possible existence of at least one other (Fig. 2C). In addition, the DGAT2 proteins contain three potential N-linked glycosylation sites, six potential protein kinase C phosphorylation sites (PROSITE data base), and sequences that share similarity with elements of a putative glycerol-phospholipid domain (GenBank access number PS50239) found in glycerol-3-phosphate acyltransferase, lysophosphatidic acid acyltransferases, and related acyltransferases (24, 25) (Fig. 2B). The latter domain is thought to be involved in the active site of the acyltransferase reaction (25). The alignment of the predicted proteins encoded by the mammalian DGAT2-related cDNAs reveals regions of identity or high similarity throughout the molecules, including many residues dispersed throughout the proteins that are conserved in all members of the family (Fig. 3).

**Characterization of DGAT2 Activity**—To determine whether DGAT2 cDNA encoded a protein possessing DGAT activity, we expressed FLAG-tagged versions of human or mouse DGAT2 in Sf9 insect cells. Insect cells were infected with recombinant mouse DGAT2 baculovirus, and membranes were isolated at different times after infection. The expression of the FLAG-tagged DGAT2 protein (molecular mass: ∼44.5 kDa) was maximal 48 h after infection (Fig. 4A, inset). In DGAT assays performed with 5 mM MgCl₂ in the assay buffer, DGAT activity in DGAT2-infected membranes was 4–6-fold greater than the endogenous activity, and the maximal activity correlated with maximal protein expression (Fig. 4A). However, when the assay contained 100 mM MgCl₂, there was no detectable increase in DGAT activity in membranes expressing DGAT2 as compared with the endogenous activity. DGAT activity was maximal for DGAT2 when 20 mM MgCl₂ was used in the assay (not shown). In a separate experiment, we expressed human DGAT2 cDNA in insect cells; human DGAT2 also catalyzed triglyceride synthesis 30-fold over background (640 versus 20 pmol/mg of protein/min with 20 mM MgCl₂ in the assay buffer) in membrane assays.

Several characteristics of DGAT2 activity identified it as a DGAT. First, triglycerides were the major product of the in vitro reaction catalyzed by DGAT2 expressed in insect cell membranes; autoradiography of TLC plates used to analyze reaction products did not reveal the incorporation of [14C]-oleoyl-CoA substrate into other lipids (not shown). Second, DGAT2 activity was primarily localized in the membrane fraction (data not shown). Third, membranes expressing DGAT2²

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² If this is the case, we propose to name the gene described in this paper DGAT2a and subsequent DGATs in this gene family DGAT2b, DGAT2y, etc. This will distinguish this family of DGAT genes from DGAT1, which is a member of the ACAT/DGAT gene family.
catalyzed a 2-fold increase in triglyceride synthesis over endogenous activity (354 ± 54 versus 169 ± 42 pmol/mg protein/min, n = 3) when labeled diacylglycerol was used as a substrate. In addition, adding exogenous diacylglycerol stimulated DGAT activity 10-fold in membranes expressing DGAT2, establishing the dependence on diacylglycerol as a substrate (Fig. 4B). Fourth, the mass of triglycerides produced from the in vitro reaction increased as a function of the oleyl-CoA concentration in membranes expressing FLAG-tagged DGAT2 (Fig. 4C). Triglyceride synthesis by DGAT2 was similar to that for comparable levels of expressed FLAG-tagged DGAT1, although at high concentrations of oleyl-CoA (200 μM), DGAT1 retained 80% of its maximal activity, whereas DGAT2 retained only 25%. Finally, we tested membranes expressing DGAT2 for activities with other fatty acyl-CoA acceptors, including glycerol 3-phosphate, lysophosphatic acid, and monoacylglycerol. Activity was only found when sn-1,2-diacylglycerol was used (Fig. 4D).

**Fig. 4.** Expression of DGAT2 cDNA in insect cells. A, correlation of DGAT activity with DGAT2 protein expression. Sf9 insect cells were infected with baculoviruses containing wild-type sequences or the FLAG-tagged mouse DGAT2 cDNA (DGAT2). Cells were collected at different times after infection, and the membrane protein fraction was assayed for DGAT activity by using [14C]oleoyl-CoA and exogenous diacylglycerol in acetone as substrates. Assays were performed with 5 or 100 mM MgCl₂. The data are duplicate values for one experiment, which was repeated with similar results. DGAT2 protein expression levels were analyzed by immunoblotting with an anti-FLAG antibody (inset). B, dependence of DGAT2-catalyzed DGAT activity on diacylglycerol. Insect cell membranes expressing wild-type baculovirus proteins, mouse DGAT1, or mouse DGAT2 were assayed for DGAT activity using [14C]oleoyl-CoA with or without exogenous diacylglycerol substrate (DAG). The assay mix contained 20 mM MgCl₂. Results are the mean ± S.D. of two experiments. C, dependence of DGAT2-catalyzed DGAT activity on oleyl-CoA. DGAT activity was compared for insect cell membranes expressing similar levels of mouse DGAT1 or DGAT2 (as determined by immunoblotting for the FLAG epitope) over a range of oleyl-CoA concentrations. The assay mix contained 0.4 mM diacylglycerol and 20 mM MgCl₂. Triglyceride mass was quantified by scanning densitometry. Data are expressed as arbitrary units/mg of protein/min. The experiment was repeated once with similar results. D, utilization of diacylglycerol but not other fatty acyl-CoA acceptors in assays of membranes expressing DGAT2. Insect cell membranes expressing LAcZ control baculovirus or DGAT2 were incubated with [14C]oleoyl-CoA and different acyl-CoA acceptors as described under “Materials and Methods.” Membranes isolated from wild-type mouse liver was used as positive controls for different activities. Note that DGAT2 utilized diacylglycerol (DAG) as a substrate for incorporation of label into triglyceride (TG) (lane 5) but did not use glycerol 3-phosphate (G-3P) for lysophosphatic acid (LPA) synthesis (lane 8), lysophosphatic acid for phosphatidic acid synthesis (lane 11), or sn-2-monoacylglycerol (2-MAG) for diacylglycerol synthesis (lane 14). When LPA was used as the acyl acceptor, more labeled phosphatidic acid accumulated in membranes expressing wild-type virus (lane 10) than those expressing DGAT2 (lane 11). This result was obtained in three separate experiments and presumably reflects the utilization of phosphatidic acid as a precursor for diacylglycerol substrate in the DGAT reaction when DGAT2 is present in large amounts. wt, wild type.
Next, we examined fatty acyl-CoA substrate specificity for DGAT1 and DGAT2 by comparing the ability of palmitoyl-CoA, linoleoyl-CoA, and arachidonyl-CoA to compete with labeled oleoyl-CoA in DGAT assays. For both DGAT1 and DGAT2, arachidonyl-, linoleoyl-, and palmitoyl-CoA competed similarly with oleoyl-CoA for incorporation into triglycerides (Table I).

Tissue Expression of DGAT2—Northern blots of human and mouse tissues were performed to determine whether DGAT2 is expressed in tissues that are relevant to triglyceride metabolism. Two mRNA species of 2.4 and 1.8 kilobases were detected for human DGAT2 (Fig. 4A). The significance of the two mRNA species is unknown. In human tissues, the highest levels of expression were in liver and white adipose tissue. Lower levels of expression were found in mammary gland, testis, and peripheral blood leukocytes (Figs. 5, A and B). The high expression levels in mammary gland may result in part from expression in adipocytes. In mouse tissues, the major mRNA for DGAT2 was 2.4 kilobases in size (Fig. 5C). An additional smaller hybridization band was observed in mouse testis RNA. The pattern of expression was generally similar in mouse and human tissues, except in small intestine, where DGAT2 expression was found in mice, but was very low in humans in three independent Northern blots. DGAT2 was also expressed at high levels in brown adipose tissue in mice (Fig. 5D). DGAT2 mRNA levels were not up-regulated in liver or adipose tissues of Dgat<sup>−/−</sup> (DGAT1-deficient) mice (Fig. 5D).

We also examined DGAT2 expression in a cultured cell model of adipocyte differentiation (NIH 3T3-L1 cells) in which DGAT activity increases markedly, both in the presence and absence of MgCl<sub>2</sub> in the assay mix (Refs. 8 and 25 and data not shown). After 10 days of differentiation, DGAT2 mRNA increased 30-fold, consistent with a possible role in adipogenesis (Fig. 6).

**DISCUSSION**

The disruption of the mouse DGAT1 gene indicated the existence of multiple mechanisms for triglyceride synthesis (12). In the current study, we cloned and characterized DGAT2, a second mammalian DGAT that was identified by its homology to a DGAT in *M. rammaniana.*<sup>2</sup> DGAT2 is a member of a family of genes whose members are unrelated to DGAT1 by sequence homology and are candidate genes for additional mammalian DGATs. DGAT1 and DGAT2 exhibit different sensitivities to MgCl<sub>2</sub> in *in vitro* assays, and the activity of DGAT2 may account for the residual DGAT activity in *Dgat<sup>−/−</sup>* mice, which is apparent when lower concentrations (0–20 mM) of MgCl<sub>2</sub> are used in the assay.

Several findings indicate that the DGAT2 cDNA encodes a DGAT. First, overexpression of this cDNA in insect cell membranes resulted in large increases in triglyceride synthesis in *in vitro* assays, as measured by either accumulation of mass or radiolabeled tracer. Second, increases in triglyceride synthesis activity were directly related to increases in the concentration of either substrate, diacylglycerol or oleoyl-CoA, in the reaction. Third, DGAT2 catalyzed the synthesis of labeled triglyceride formed per mg of protein/min; for DGAT2, it was 4880 pmol of triglyceride formed per mg of protein/min; for DGAT1, it was 6850 pmol of triglyceride formed per mg of protein/min. For both DGAT1 and DGAT2, arachidonyl-, linoleoyl-, and palmitoyl-CoA competed similarly with oleoyl-CoA in DGAT assays. For both DGAT1 and DGAT2, the activity with oleoyl-CoA alone was 6850 pmol of triglyceride formed per mg of protein/min; for DGAT2, it was 4880 pmol of triglyceride formed per mg of protein/min.

| Protein expressed | Relative activities | Oleyl-CoA | Palmitoyl-CoA | Arachidonyl-CoA | Linoleoyl-CoA |
|------------------|--------------------|----------|--------------|----------------|--------------|
| DGAT1            | 1.00               | 0.67     | 0.49         | 0.44           | 0.54         |
| DGAT2            | 1.00               | 0.46     | 0.45         | 0.44           | 0.44         |

**FIG. 5. Tissue expression pattern of DGAT2.** A, DGAT expression in a human Multiple Tissue Northern blot (CLONTECH). A 3′ partial human DGAT2 cDNA fragment was used as a probe. The adipose tissue sample is from a different multiple tissue blot (Invitrogen). Sm., small. Periph. Leuk., peripheral leukocytes. B, DGAT expression in a human Multiple Tissue Expression Array (CLONTECH). Relative expression levels were quantified with a phosphorimager (Fuji). Expression levels for human small intestine were very low and are not shown in the figure. PG, paracentral gyrus. C, DGAT expression in a mouse Multiple Tissue Northern blot (CLONTECH). A 3′ partial mouse DGAT2 cDNA fragment was used as a probe. Sm., smooth. Sk., skeletal. D, DGAT expression in tissues of wild-type and *Dgat<sup>−/−</sup>* mice. WAT, white adipose tissue; BAT, brown adipose tissue. The autoradiograms were exposed for about 18 h.
mid-range (insect cells enabled us to compare the activity levels and sub-
thesis is the sole function of this enzyme. Experiments will be useful in determining whether triglyceride
acids using either oleoyl-CoA or diacylglycerol as the labeled
zymes are not rate-limiting for triglyceride synthesis and that
ative for the residual DGAT activity in Dgat−/− tissues. The observation that DGAT2 activity is max-
and MgCl2 concentrations are low supports this possibility, as DGAT activities in Dgat−/− tissues
rates of oleoyl-CoA concentration. DGAT2 tended to be slightly more active at lower oleoyl-CoA concen-
theses that do not share obvious homology. Interestingly,
ents have different capacities for triglyceride
zymes have different activities and Northern blots were performed with a mouse DGAT2 probe. Signals were quantified with a phosphorimager (Fuji), and fold increases were calculated after normal-
ing for RNA loading with actin.
Dgat2 mRNA expression in adipocyte differentiation of NIH 3T3-L1 cells. Total RNA from 3T3-L1 cells was isolated at different stages of adipocyte differentiation, and Northern blots were performed with a mouse DGAT2 probe. Signals were quantified with a phosphorimager (Fuji), and fold increases were calculated after normal-
Dgat2 clone was isolated from NIH 3T3-L1 cells. Total RNA from 3T3-L1 cells was isolated at different stages of adipocyte differentiation, and Northern blots were performed with a mouse DGAT2 probe. Signals were quantified with a phosphorimager (Fuji), and fold increases were calculated after normal-
DGAT2 activity in Dgat−/− mice. It is unclear why the residual DGAT activity in Dgat−/− tissues, which presumably is due to DGAT2 or other DGAT2 gene family members, is relatively low, even when low MgCl2 concentrations are used in the assay. It is possible that our enzyme assays have not been fully optimized to detect DGAT2 activity. It is also possible that DGAT en-
zymes are not rate-limiting for triglyceride synthesis and that
level of activity is not crucial for regulating this process.
The identification of a second DGAT in humans and mice has signif-
icant implications for the understanding of triglyceride synthesis and metabolism. Molecular tools will now be available to study the regulation, localization, and cell biology of the two enzymes and to elucidate their respective roles in physiol-
and human disease.

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