High-content assays for evaluating cellular and hepatic diacylglycerol acyltransferase activity

Jenson Qi,* Wensheng Lang,* Edward Giardino,* Gary W. Caldwell,* Charles Smith,* Lisa K. Minor,* Andrew L. Darrow,* Gustaaf Willemsens,† Katharina DeWaepenaert,† Peter Roevens,† Joannes T. M. Linders,† Yin Liang,* and Margery A. Connelly*

Johnson and Johnson Pharmaceutical Research and Development, LLC, Spring House, PA* and Beerse, Belgium†

Abstract Acyl-CoA:diacylglycerol acyltransferase (DGAT) catalyzes the terminal step in triglyceride (TG) synthesis using diacylglycerol (DAG) and fatty acyl-CoA as substrates. In the liver, the production of VLDL permits the delivery of hydrophobic TG from the liver to peripheral tissues for energy metabolism. We describe here a novel high-content, high-throughput LC/MS/MS-based cellular assay for determining DGAT activity. We treated endogenous DGAT-expressing cells with stable isotope-labeled [13C18]oleic acid. The [13C18]oleoyl-incorporated TG and DAG lipid species were profiled. The TG synthesis pathway assay was optimized to a one-step extraction, followed by LC/MS/MS quantification. Further, we report a novel LC/MS/MS method for tracing hepatic TG synthesis and VLDL-TG secretion in vivo by administering [13C18]oleic acid to rats. The [13C18]oleic acid-incorporated VLDL-TG was detected after one-step extraction without conventional separation of TG and recovery by derivatizing [13C18]oleic acid for detection. Using potent and selective DGAT1 inhibitors as pharmacological tools, we measured changes in [13C18]oleoyl-incorporated TG and DAG and demonstrated that DGAT1 inhibition significantly reduced [13C18]oleoyl-incorporated VLDL-TG. This DGAT1-selective assay will enable researchers to discern differences between the roles of DGAT1 and DGAT2 in TG synthesis in vitro and in vivo. —Qi, J., W. Lang, E. Giardino, G. W. Caldwell, C. Smith, L. K. Minor, A. L. Darrow, G. Willemsens, K. DeWaepenaert, P. Roevens, J. T. M. Linders, Y. Liang, and M. A. Connelly. High-content assays for evaluating cellular and hepatic diacylglycerol acyltransferase activity. J. Lipid Res. 2010. 51: 3559–3567.

Triglycerides (TGs) are the chief route of transport of dietary fat, within chylomicrons and VLDL, as well as the main form of fuel storage in adipose tissue. In addition, TGs play an important role in metabolism due to the fact that they are a major source of energy. TGs are synthesized from glycerol and three FA molecules; each FA is attached via an ester bond to hydroxyl groups of the glycerol backbone. Like many neutral lipids, TGs contain FA molecules with varying chain lengths; the most common are 16, 18, or 20 carbons. The two major biosynthetic pathways of TG are the glycerol-3-phosphate pathway, which exists primarily in liver and adipose tissues, and the monoacylglycerol pathway, which exists predominately in the intestine. The final step of the glycerol-3-phosphate biosynthetic pathway can be catalyzed by either diacylglycerol acyltransferase 1 (DGAT1) or DGAT2 (1, 2). Although DGAT1 and DGAT2 both convert diacylglycerol (DAG) to TG, they do not share similarity in either their nucleotide or amino acid sequences.

It has been reported that knockout mice lacking DGAT1 (DGAT1<sup>−/−</sup>) do not display obvious changes in TG metabolism in the liver (3). In contrast, knockout mice lacking DGAT2 (DGAT2<sup>−/−</sup>) display severely reduced TG content in the liver (4). Furthermore, studies have shown that suppression of DGAT2 with antisense oligonucleotides reduced hepatic TG content in rodents (5, 6) producing reversed diet-induced hepatic steatosis and insulin resistance in rats (5). These results suggest that DGAT1 and DGAT2 function differently in TG biosynthesis. The finding that multiple enzymes catalyze the synthesis of TG from DAG presents an opportunity to modulate one catalytic mechanism of this biochemical reaction. This type of modulation may produce therapeutic results without the potential adverse side effects that might occur if TG synthesis were completely inhibited in all tissues. By specifically inhibiting the activity of DGAT1 or DGAT2, compounds that inhibit the conversion of DAG to TG will be useful in lowering absorption and circulating concentrations of TG.

Supplementary key words [13C18]oleic acid • triglyceride • glycerol-3-phosphate pathway • liquid chromatography/tandem mass spectrometry • high-content assay

Abbreviations: apoB, apolipoprotein B; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; FAF-BSA, FA-free BSA; MTP, microsomal triglyceride transfer protein; SCD-1, steryl-CoA desaturase 1; TG, triglyceride.

*To whom correspondence should be addressed: e-mail: jqi@its.jnj.com
This reduction could therapeutically counteract the pathogenic effects caused by abnormal lipid metabolism in obesity, metabolic syndrome, type II diabetes, and atherosclerosis.

Conventional in vitro assays for measuring cellular TG synthesis use radiolabeled substrates and are generally performed in a 6- or 12-well format. Furthermore, the product of the ultimate, DGAT-mediated step in the biosynthetic pathway is typically resolved by thin-layer chromatography (TLC) or by using a cumbersome organic solvent extraction procedure (4, 7, 8). Therefore, there is a need to develop a high-throughput, high-content in vitro cell-based DGAT assay. Moreover, stable isotope-labeled glycerol and palmitate have been used to measure the kinetics of VLDL-TG secretion and metabolism in vivo (9, 10). However, these methods require isolation of VLDL-TG by TLC and necessitate hydrolysis of the samples in order to release the glycerol/palmitate. Labeled VLDL-TG is typically quantified by indirect measurement of the derivatized stable isotope-labeled glycerol/palmitate by GC-MS. Here we describe a novel, high-throughput LC/MS/MS-based cellular assay for detecting newly synthesized TG using endogenously expressing DGAT1 cells and a stable isotope-labeled oleic acid. In addition to interrogating the last step in TG synthesis, this high-content assay allows profiling of other intermediate steps within the glycerol-3-phosphate pathway. Furthermore, to probe alterations in TG synthesis in vivo, we administered a bolus of stable isotope-labeled oleic acid to rats. The major species of stable isotope-labeled oleoyl-incorporated TG from plasma were detected by LC/MS/MS after a once-sample extraction in high-throughput format. Using this assay, we explored changes in newly synthesized TG in cells endogenously expressing DGAT1, and examined the activity of a DGAT1 inhibitor on newly synthesized and secreted VLDL-TG in vivo.

MATERIALS AND METHODS

Materials and reagents

Ammonium formate, triolein, FA-free BSA (FABSA), [13C15] oleic acid, and oleic acid were purchased from Sigma-Aldrich (St. Louis, MO). Diolein, and 1,3-di-heptadecanoyl-2-(10Z-heptadecanoyl)-ω-ω-glycerol-d5 were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). Isopropyl alcohol, acetonitrile, and tetrahydrofuran were from EMD Chemicals, Inc. (Gibbstown, NJ). DGAT1-selective inhibitor A-922500 (Abbott), chemical name (1R,2R)-2-[(4'-[[phenylamino]carbonyl]amino)[1,1'-biphenyl]-4-yl]carbonyl)cyclopentanecarboxylic acid, was purchased from Tocris Bioscience (Ellisville, MO). JNJ compound A, chemical name N-[2,6-dichloro-4-[pyrrolidin-1-ylmethyl] phenyl]-4-(4-[4-(4-methoxyphenyl)acetyl]amino)phenyl)piperazine-1-carboxamide), was synthesized by in-house chemists.

In vitro assays for recombinant hDGAT1 and hDGAT2 activity

The determination of recombinant human DGAT1 (hDGAT1) or hDGAT2 activity was readily achieved using a high-throughput screening FlashPlate assay. In this assay, recombinant hDGAT1 or hDGAT2 was produced in the baculovirus expression system.
Metabolic stable isotope labeling of the TG pathway in HEK293 cells

After one washing with PBS (pH 7.4), the confluent HEK293 cells were incubated with DMEM supplemented with 25 mM HEPEES (pH 7.5) and 0.2% FAF-BSA at 37°C/5% CO₂ for 60 min. DGAT1 inhibitors were added to cells in a concentration-dependent manner to a final concentration of 0.2% DMSO/vehicle in DMEM supplemented with 25 mM HEPEES (pH 7.5) and 0.2% FAF-BSA. Control wells received 0.2% DMSO without compound. Cells were incubated with 0.2% DMSO or compound at 37°C/5% CO₂ for 15 min. [13C18]oleic acid, complexed with FAF-BSA, was added to each well at a final concentration of 150 μM. The plates were incubated for 2 h at 37°C, and the medium was removed. After brief air-drying, 100 μl freshly prepared extract solvent (90% isopropl alcohol and 10% tetrahdyroforan, containing 20 nM of the internal standard [1,3-dii-heptadecanoyl-2-(10Z-heptadecanoyl)-sn-glycerol-d5], was added and allowed to stand at room temperature for 15 min with shaking. The extraction mixture (80 μl) was transferred to glass inserts in a 96-well deep-well plate. The plate was centrifuged at 3,000 rpm for 5 min, and the extracted samples were analyzed via LC/MS/MS. Percentage inhibition was calculated as 100 – [(sample – low control) / (vehicle – low control) × 100], where low control is no addition of [13C18]oleic acid. A best-fit curve is fitted by a minimum sum-of-squares method to the plot of percent inhibition versus compound concentration in GraphPad Prism software.

RESULTS AND DISCUSSION

The conventional cell-based TG synthesis assay uses radiolabeled FAs to metabolically label TG molecules. The radiolabeled hydrophobic TG product is typically resolved by TLC (4, 7, 8). However, LC/MS/MS is becoming the preferred method for quantitative lipid analysis due to the ease of automation, accuracy, and sensitivity, and the avoidance of radioactivity. Because triolein is the most-abundant TG in mammals and oleoyl-CoA may be the most-physiologically relevant acyl donor for the DGAT reaction, stable isotope-labeled oleic acid ([13C18]oleic) was chosen to metabolically trace DAG and TG synthesis. After loading the cells with [13C18]oleic acid complexed with FAF-BSA, [13C18]oleic acid was converted to [13C18]oleyl-CoA (H₁₈C₁₈-CO-S-CoA) before entering the glycerol-3-phosphate pathway (Fig. 1). Multiple conditions were tested before choosing a suitable solvent (e.g., 90% isopropl alcohol and 10% tetrahdyroforan) to extract neutral lipids from the cell monolayer in a 96-well format. This solvent mixture efficiently extracts neutral lipids, similar to the standard chloroform/methanol method (data not shown), and is compatible with direct LC/MS/MS analysis without solvent exchange.

Analysis of cell-based [13C18]oleic acid incorporation into DAG and TG

The [13C18]oleic acid-incorporated DAGs and TGs in HEK293 cells were extracted and analyzed by LC/MS/MS (Fig. 2). The triolein species containing three, two, or one [13C18]oleoyl side chains were designated as triolein ([13C18]oleoyl, [13C18]oleoyl, [13C18]oleoyl), triolein ([13C18]oleoyl, [13C18]oleoyl, oleoyl), and triolein ([13C18]oleoyl, oleoyl, oleoyl).
Oleoyl, oleoyl, oleoyl), respectively (Fig. 2A). In addition, 

\[^{13}C_{18}\]oleoyl-incorporated DAGs were also analyzed in a separate run. Two major \[^{13}C_{18}\]-labeled DAGs, diolein (\[^{13}C_{18}\]oleoyl, \[^{13}C_{18}\]oleoyl) and diolein (\[^{13}C_{18}\]oleoyl, oleoyl), along with endogenous diolein (oleoyl, oleoyl) were detected (Fig. 2B). The particular position of the \[^{13}C_{18}\]oleoyl or oleoyl group in the side chain of triolein or diolein is not distinguishable using LC/MS/MS.

We determined the time course of \[^{13}C_{18}\]oleoyl incorporation into TG in HEK293 cells using 150 \(\mu\)M and 300 \(\mu\)M of \[^{13}C_{18}\]oleic acid (Fig. 3). \[^{13}C_{18}\]oleic acid concentrations were consistent with the concentration of exogenous oleic acid used to stimulate cellular TG synthesis (13, 14). Under our experimental conditions, three \[^{13}C_{18}\]oleoyl-incorporated triolein, designated as triolein (\[^{13}C_{18}\]oleoyl, \[^{13}C_{18}\]oleoyl, \[^{13}C_{18}\]oleoyl), \[^{13}C_{18}\]oleoyl, \[^{13}C_{18}\]oleoyl, \[^{13}C_{18}\]oleoyl), and \[^{13}C_{18}\]oleoyl, \[^{13}C_{18}\]oleoyl, oleoyl) were detected (Fig. 3A, B, C). Because the three different \[^{13}C_{18}\]oleoyl-incorporated triolein species were not available to be used as reference standards, we used unlabeled triolein to determine its concentration.
Cellular and hepatic DGAT assays

incorporated diolein ([13C18]oleoyl, [13C18]oleoyl) rose significantly at 30 min and then increased at a slower rate (Fig. 4A). In contrast, [13C18]oleoyl-incorporated triolein rose only slightly at 30 min and then increased at a faster rate following this initial lag (Fig. 3A). Taken together, these results suggested that newly synthesized diolein ([13C18]oleoyl, [13C18]oleoyl) was rapidly converted to triolein and did not accumulate to any extent.

In vitro profile of two DGAT1-selective inhibitors

JNJ compound A, chemical name N-[2,6-dichloro-4-(pyrrolidin-1-ylmethyl)phenyl]-4-[[4-(4-methoxyphenyl)acetyl]amino]phenyl]piperazine-1-carboxamide, was discovered in-house as a potent selective DGAT1 inhibitor (Table 1) (15). The IC50 of human recombinant DGAT1 is 0.019 ± 0.004 μM; the IC50 of human recombinant DGAT2, dog MTP, human recombinant ACAT2, human recombinant stearoyl-CoA desaturase 1 (SCD-1), the cellular apoB secretion assay, the human recombinant peroxisome proliferator-activated receptor α (PPARα) and PPARγ, were all greater than 10 μM (Table 1). In addition, Abbott Laboratories has reported a highly potent and selective DGAT1 inhibitor, A-922500 (16), that has an IC50 of 0.007 μM when tested against human recombinant DGAT1.
TABLE 1. In vitro activity of JNJ compound A

| DGAT1 Inhibitor | DGAT1 IC_{50} (μM) | DGAT2 IC_{50} (μM) | ACAT1 IC_{50} (μM) | ACAT2, MTP, ApoB, PPARα, PPARγ IC_{50} (μM) |
|-----------------|------------------|------------------|------------------|---------------------------------------------|
| JNJ compound A  | 0.019            | >10              | 1                | >10                                         |

Activity was determined in cell-free recombinant human diacylglycerol acyltransferase 1 (DGAT1), human DGAT2, human ACAT1, human ACAT2, microsomal triglyceride transfer protein (MTP), the peroxisome proliferator-activated receptor α (PPARα), PPARγ, stearoyl-CoA desaturase 1 (SCD-1), and apolipoprotein B (apoB) secretion assays. The human recombinant DGAT1 and DGAT2 membrane assays were performed as described in Materials and Methods. No inhibition of greater than 50% was observed against any of the receptors, ion channels, or enzymes at 10 μM of JNJ compound A when tested against a panel of cell surface receptors (www.cerep.com).

Inhibition of cell-based DGAT activity

During assay development, we tested two different chemotypes of potent and selective DGAT1 inhibitors, [N] compound A and the commercially available compound A-922500 (16). The IC_{50} values for these inhibitors in the cell-free recombinant human DGAT1 enzyme assay were 19 nM (Table 1) and 7 nM (16), respectively. Both were devoid of DGAT2 inhibitory activity (15, 16). Inhibition of cellular DGAT1 activity by the two DGAT1 inhibitors was concentration dependent as measured by the percentage decrease in the production of three [13C_{18}]oleoyl-incorporated triolein, two [13C_{18}]oleoyl-incorporated trioleins, and one [13C_{18}]oleoyl-incorporated triolein (Fig. 5). We calculated the IC_{50} values based on the percentage of inhibition for each labeled triolein using the standard Prism curve-fitting program (Fig. 5). The IC_{50} values for [13C_{18}] oleoyl triolein incorporation in this cell-based assay for JNJ compound A and A-922500 were 21 nM and 17 nM, respectively.

We then determined the [13C_{18}] oleoyl-incorporated diolein levels in the same samples as exhibited in Fig. 5. Because the signal and abundance of one [13C_{18}] oleoyl-incorporated diolein were considerably lower than those of two [13C_{18}] oleoyl-incorporated dioleins, we plotted the LC/MS/MS response of diolein ([13C_{18}] oleoyl, [13C_{18}]oleoyl) in cells treated with either JNJ compound A or A-922500. Both JNJ compound A and A-922500 increased the level of two [13C_{18}] oleoyl-incorporated dioleins in a concentration-dependent fashion (Fig. 6). The EC_{50} values calculated from the increase over the vehicle control were approximately 25 nM and 10 nM for JNJ compound A and A-922500, respectively. Interestingly, the EC_{50} values were close to the IC_{50} values determined from the inhibition of [13C_{18}] oleoyl-incorporated triolein (Fig. 5), suggesting that JNJ diolein. All mass units are relative to an internal standard. Values represent the mean of three data points ± SEM.
Analysis of $^{[13}C_{18}]$oleic acid incorporation into VLDL-TGs in vivo

We extended the tracing of TG synthesis in vivo by administering a bolus dose of $^{[13}C_{18}]$oleic acid to rats and analyzing plasma samples for $^{[13}C_{18}]$oleoyl incorporation into VLDL-TG by LC/MS/MS analysis. Two major species of $^{[13}C_{18}]$oleoyl-incorporated TGs were detected after a one-step sample extraction procedure. The two TG species that accounted for greater than 90% of total $^{[13}C_{18}]$oleoyl-incorporated TGs were TG (palmitoyl, oleoyl, $^{[13}C_{18}]$oleoyl) and TG (oleoyl, oleoyl, $^{[13}C_{18}]$oleoyl). Within 15 min after intravenous dosing of $^{[13}C_{18}]$oleic acid, $^{[13}C_{18}]$oleoyl was already efficiently incorporated into VLDL-TG. The $^{[13}C_{18}]$oleoyl-incorporated VLDL-TG peaked at 30 min (Fig. 8). A similar time-dependent curve was observed after bolus intravenous injection of $^{[13}C_{18}]$oleic acid to conscious rats through the tail vein (data not shown). Stable isotopically labeled palmitate has been used to measure newly synthesized VLDL-TG secretion and turnover in humans (9, 10). In those studies, stable

To confirm that HEK293 cells express DGAT1, we assessed the expression of DGAT1 protein by Western blot. A DGAT1 band corresponding to the size of $\sim$54 kDa was detected in HEK293 cells. On the other hand, DGAT2 was not detected in these cells (Fig. 7). MCF7 cells were used as a positive control in the Western blot because immunohistochemistry using the same anti-DGAT2 antibody showed that DGAT2 expression was very low in HEK293 cells and high in MCF7 cells (the Swedish Human Proteome Resource, www.proteinatlas.org). Therefore, our results suggest that DGAT1 is the major form of DGAT expressed in HEK293 cells and is most likely responsible for the majority of TG synthesis.
isotope-labeled palmitoyl-incorporated VLDL-TG peaked around 30 min after bolus intravenous injection (9, 10). We also measured the endogenous plasma TG level in the same samples during the experimental time frame. No significant change in the endogenous plasma TG level was detected (data not shown).

In addition to plasma, we detected the incorporation of
$[^{13}\text{C}_{18}]$oleic acid into TG in the liver during this acute study. Liver and adipose TG levels were measured 1 h after $[^{13}\text{C}_{18}]$oleic acid administration. The percentage of $[^{13}\text{C}_{18}]$oleic acid-incorporated TG was $\sim0.2\%$ of the total endogenous hepatic TG pool versus $\sim2\%$ in the plasma. However, $[^{13}\text{C}_{18}]$incorporated TG in the adipose tissue was barely detected at 1 h, due to very high levels of endogenous TG present in this tissue. In addition, most organic compounds distribute to adipose tissue more slowly than to the liver. Therefore, sub-chronic $[^{13}\text{C}_{18}]$oleic acid infusion may be required to trace DGAT activity in tissues such as heart, adipose, and skeletal muscle. Further studies are necessary to evaluate the utility of this assay for studying the distribution of newly synthesized TG with LC/MS/MS.

**Inhibition of $[^{13}\text{C}_{18}]$oleic acid incorporation into VLDL-TGs in vivo**

Mammals have two DGAT enzymes, DGAT1 and DGAT2, that are members of distinct gene families (1, 2). Although both are highly expressed in the liver, only DGAT2 is thought to be involved in the bulk of TG synthesis, such that it may be closely linked with the pathways of de novo FA biosynthesis (17, 18). In contrast, cumulative evidence suggests that DGAT1 may be involved in esterifying exogenous FAs taken up by cells or in a recycling pathway that involves the reesterification of hydrolyzed TG (17, 19, 20). To determine whether DGAT1 is directly responsible for the esterification of exogenous oleic acid, we examined the DGAT1-selective inhibitor [NJ compound A in the hepatic synthesis and secretion of $[^{13}\text{C}_{18}]$oleoyl-incorporated VLDL-TG in rats. In fact, subcutaneous injection of [NJ compound A at 3 mg/kg significantly blocked newly synthesized $[^{13}\text{C}_{18}]$oleoyl-incorporated VLDL-TG (Fig. 9). The plasma levels of [NJ compound A were 0.07 ± 0.01 μM and 0.10 ± 0.01 μM at 30 min or 60 min post $[^{13}\text{C}_{18}]$oleic acid injection, respectively. These concentrations were higher than the IC$_{50}$ of [NJ compound A in the cellular assay (Fig. 5A), suggesting that we had indeed reached compound exposure levels high enough to effectively inhibit DGAT1 in vivo. Taken together, our results support the hypothesis that DGAT1 is directly involved in the synthesis of hepatic TG from exogenous FA.

In conclusion, we have developed a novel, high-content assay for the evaluation of cellular and hepatic DGAT activity without the use of radioactive precursors. Our results provide additional evidence to support the functional role of DGAT1 in cellular and hepatic TG synthesis pathways. These methods will be valuable tools for high-throughput screening efforts to discover DGAT inhibitors as well as to interrogate the effects of DGAT inhibitors in vitro and in vivo.

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**REFERENCES**

1. Cases, S., S. J. Smith, Y. W. Zheng, H. M. Myers, S. R. Lear, E. Sande, S. Novák, C. Collins, C. B. Welch, A. J. Lusis, et al. 1998. Identification of a gene encoding an acyl-CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. Proc. Natl. Acad. Sci. USA. 95: 13018–13023.
2. Cases, S., S. J. Stone, P. Zhou, E. Yen, B. Tow, K. D. Lardizabal, T. Voelker, and R. W. Farese. Jr. 2001. Cloning of DGAT2, a second mammalian diacylglycerol acyltransferase, and related family members. J. Biol. Chem. 276: 38870–38876.
3. Smith, S. J., S. Cases, D. R. Jensen, H. C. Chen, E. Sande, B. Tow, D. A. Sanan, J. Raber, R. H. Eckel, and R. W. Farese. Jr. 2000.
Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nat. Genet.* **25**: 87–90.

4. Stone, S. J., H. M. Myers, S. M. Watkins, B. E. Brown, K. R. Feingold, P. M. Elias, and R. W. Farese, Jr. 2004. Lipopedia and skin barrier abnormalities in DGAT2-deficient mice. *J. Biol. Chem.* **279**: 11767–11776.

5. Choi, C. S., D. B. Savage, A. Kulkarni, X. X. Yu, Z. X. Liu, K. Morino, S. Kim, A. Distefano, V. T. Samuel, S. Neschen, et al. 2007. Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverses diet-induced hepatic steatosis and insulin resistance. *J. Biol. Chem.* **282**: 22678–22688.

6. Yu, X. X., S. F. Murray, S. K. Pandey, S. L. Booten, D. Bao, X. Z. Song, S. Kelly, S. Chen, R. McKay, B. P. Monia, et al. 2005. Antisense oligonucleotide reduction of DGAT2 expression improves hepatic steatosis and hyperlipidemia in obese mice. *Hepatology*. **42**: 362–371.

7. Bruce, J. S., and A. M. Salter. 1996. Metabolic fate of oleic acid, palmitic acid and stearic acid in cultured hamster hepatocytes. *Biochem. J.* **316**: 847–852.

8. Cheng, D., J. Iqbal, J. Devenny, C. H. Chu, L. Chen, J. Dong, R. Seethala, W. J. Keim, A. V. Azzara, R. M. Lawrence, et al. 2008. Acylation of acylglycerols by acyl coenzyme A diacylglycerol acyltransferase 1 (DGAT1). Functional importance of DGAT1 in the intestinal fat absorption. *J. Biol. Chem.* **283**: 29802–29811.

9. Patterson, B. W., B. Mittendorfer, N. Elias, R. Satyanarayana, and S. Klein. 2002. Use of stable isotopically labeled tracers to measure very low density lipoprotein-triglyceride turnover. *J. Lipid Res.* **43**: 223–233.

10. Magkos, F., B. W. Patterson, and B. Mittendorfer. 2007. Reproducibility of stable isotope-labeled tracer measures of VLDL-triglyceride and VLDL-apolipoprotein B-100 kinetics. *J. Lipid Res.* **48**: 1204–1211.

11. Wetterau, J. R., and D. B. Zilversmitt. 1986. Localization of intracellular triacylglycerol and cholesteryl ester transfer activity in rat tissues. *Biochim. Biophys. Acta*. **875**: 610–617.

12. Ntambi, J. M. 1992. Dietary regulation of stearoyl-CoA desaturase 1 gene expression in mouse liver. *J. Biol. Chem.* **267**: 10925–10930.

13. Listenberger, L. L., X. Han, S. E. Lewis, S. Cases, R. V. Farese, Jr., D. S. Orr, and J. E. Schaffer. 2003. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc. Natl. Acad. Sci. USA*. **100**: 3077–3082.

14. Lee, J. Y., H. K. Cho, and Y. H. Kwon. 2010. Palmitate induces insulin resistance without significant intracellular triglyceride accumulation in HepG2 cells. *Metabolism*. **59**: 927–934.

15. Linders, J. I. M., P. Roevens, M. Berwaer, S. Boeckx, J-P. Bongartz, H. Bonghys, C. Buyc, E. Coesemans, P. V. Davidenko, R. Gilissen, et al. 2009 Discovery, synthesis, and in vivo activity of phenylpiperazine DGAT1 inhibitors for the treatment of metabolic syndrome. (Abstract in 238th ACS National Meeting. Washington, DC, 2009).

16. Zhao, G., A. J. Souers, M. Voorbach, H. D. Falls, B. Droz, S. Brodjian, Y. Y. Lau, R. R. Iyengar, J. Gao, A. S. Judd, et al. 2008. Validation of diacylglycerolacyltransferase I as a novel target for the treatment of obesity and dyslipidemia using a potent and selective small molecule inhibitor. *J. Med. Chem.* **51**: 380–383.

17. Man, W. C., M. Miyazaki, K. Chu, and J. Ntambi. 2006. Colocalization of SCD1 and DGAT2: implying preference for endogenous mono-unsaturated fatty acids in triglyceride synthesis. *J. Lipid Res.* **47**: 1928–1939.

18. Ven, C. L., S. J. Stone, S. Koliwad, C. Harris, and R. V. Farese, Jr. 2008. Thematic review series: glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. *J. Lipid Res.* **49**: 2283–2301.

19. Villanueva, C. J., M. Monetti, M. Shih, P. Zhou, S. M. Watkins, S. Bhaniot, and R. V. Farese, Jr. 2009. Specific role for acyl CoA:diacylglycerol acyltransferase 1 (Dgat1) in hepatic steatosis due to exogenous fatty acids. *Hepatology*. **50**: 434–442.

20. Yamazaki T., E. Sasaki, C. Kakinuma, T. Yano, S. Miura, and O. Ezaki. 2005. Increased very low density lipoprotein secretion and gonadal fat mass in mice overexpressing liver DGAT1. *J. Biol. Chem.* **280**: 21506–21514.