Title page

Drug targeting of acyltransferases in the Triacylglyceride and 1-O-AcylCeramide biosynthetic pathways

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Therapeutic Implications for Sphingolipids in Health and Disease for...

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Abbreviations:
AC: acylceramide; AGPAT: 1-acylglycerol-3-phosphate O-acyltransferase; CAT: ceramide acyltransferase; CERS: ceramide synthase; DAG: diacylglyceride; DGAT: diacylglyceride acyltransferase; GPAT: glycerol-3-phosphate acyltransferase; GPCR: G-protein coupled receptor; LPA: lysophosphatidic acid; MAMs: mitochondria-associated membranes; MOGAT: monoacylglycerol acyltransferase; NAFLD: nonalcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; PA: phosphatidic acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PG: phosphatidylycerol; PKC: protein kinase C; PI: phosphatidylinositol; PS: phosphatidylserine; SM: sphingomyelin; SPT: serine palmitoyltransferase; TAG: triacylglyceride; TNF: tumor necrosis factor; VLDL: very low density lipoprotein.
Abstract

Acyltransferase enzymes (EC 2.3.) are a large group of enzymes that transfer acyl groups to a large variety of substrates. This review focuses on fatty acyltransferases involved in the biosynthetic pathways of glycerolipids and sphingolipids and how these enzymes have been pharmacologically targeted in their biological context. Glycerolipids and sphingolipids, commonly treated independently in their regulation and biological functions, are put together to emphasize the parallelism in their metabolism and bioactive roles. Furthermore, a newly considered signaling molecule, 1-O-acylceramide, resulting from the acylation of ceramide by DGAT2 enzyme, is discussed. Finally, the implications of DGAT2 as a putative Ceramide AcylTransferase (CAT) enzyme, with a putative dual role in TAG and 1-O-acylceramide generation, are explored.
Significance Statement

This manuscript reviews the current status of drug development in lipid acyltransferases. These are current targets in metabolic syndrome and other diseases, including cancer. A novel function for a member in this group of lipids has been recently reported in cancer cells. The responsible enzyme and biological implications of this added member are discussed.
1. Introduction

According to LIPID MAPS lipid classification, we can distinguish between two large groups of lipids: Those lipids that are based on fatty acyls and the ones equivalent to the chemical condensation of isoprene units (for a better chemical detail on the classification; please visit https://lipidmaps.org/ (O’Donnell et al., 2019). Fatty acyls are a large group of thousands of distinct chemical structures that contain fatty acids, other derivatives, such as branched-chain fatty acids, and various functional groups, including esters and alcohol groups (Fahy et al., 2011). In cells, fatty acids are the building blocks of different families of lipids, such as glycerolipids, glycerophospholipids, sphingolipids, and saccharolipids. Each group consists of a characteristic backbone, where one or more fatty acids are added with the help of acyltransferases (Enzyme Commission number EC 2.3.1). Thus, glycerol, glycerol-3-phosphate, sphingosine, and sugar moieties are the backbones where fatty acids hang for the previously mentioned lipids, respectively (Turkish et al., 2005). In a cell metabolic context, lipid acylation reactions are often not linear and are organized in interconnected metabolic pathways (Canals, 2021).

Glycerolipids and sphingolipids have parallel biosynthetic pathways, where their backbone structures can be sequentially acylated with fatty acids up to reach triacylglycerides (TAGs) and 1-O-acylceramides (ACs). Their Plutarchian parallelism is kept along their biosynthesis pathway (Figure 1): The first cycle of transferase, generating lysolipids (lysophosphatidic acid, LPA, and a sphingoid base, which can also be phosphorylated) are partially soluble in an aqueous environment. These compounds are bioactive molecules with known G-protein coupled receptors (GPCR), the lysospholipid family of receptors (LPL-R), which trigger similar signaling pathways, regulating different biologies in cell motility and growth (Binder et al., 2015). The structures resulting after the second acyltransferase cycle are the main constituents of cellular membranes, and the position in C-1 is similarly modified in both groups to produce phosphocholines, such as phosphatidylcholines (PC) and sphingomyelins (SM), ethanol amines, and glycolipids. Moreover, diacylglycerol (DAG) and ceramide are signaling molecules that directly activate protein effectors, such as protein kinase C (PKC, by DAG, and ceramide) and protein phosphatases 1 and 2A (by ceramide). Specific kinases can phosphorylate both DAG and ceramide to generate bioactive molecules. Upon three acyl transferase reactions, the resulting molecules (TAGs and acylceramides) are highly hydrophobic. TAGs and acylceramides are reported to be stored in lipid droplets and are essential to maintain the skin permeability barrier function (Rabionet et al., 2013; Radner and Fischer, 2014). This review focuses on the acyltransferase enzymes leading to TAG and AC formation, the current status of the drug targeting these enzymes, and the interconnection between the two parallel pathways.

2. Acyltransferase enzymes in TAG and 1-O-acylceramide pathways

Triacylglycerides (TAGs) are synthesized through two major pathways, the glycerol phosphate pathway and the monoacylglycerol pathway. The glycerol phosphate pathway is the predominant pathway utilized by most cell types (Takeuchi and Reue, 2009). In the glycerol phosphate pathway, glycerolipids are built in a
series of sequential acyltransferase reactions using glycerol as a backbone (in the form of glycerol-3-phosphate). These enzymes are glycerol-3-phosphate acyltransferases (GPATs), 1-acylglycerol-3-phosphate O-acyltransferases (AGPAT), and diacylglycerol O-acyltransferase (DGATs) (Figure 1). It is important to note that these steps are not necessarily intermediate to generating TAGs. However, each sequentially acylated molecule also serves signaling functions (e.g., Lysophosphatidic acid or LPA, known to interact with membrane receptors, and DAG, a second messenger that activates PKC, among other enzymes) and structural roles (e.g., in the formation of phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidyserine (PS), phosphatidylglycerol (PG), phosphatic acid (PA), and phosphatidylinositol (PI)). In humans, there are four isoforms for GPAT: GPAT1/GPAM, GPAT2, GPAT3, and GPAT4, 11 isoforms for AGPAT, and two main enzymes for DGAT activity (Takeuchi and Reue, 2009). Additionally, intermediary structures in this biosynthetic pathway can originate directly from the diet and may involve other acyltransferases. Thus, in the small intestine, the monoacylglycerol pathway is the main pathway of TAG synthesis in enterocytes after a meal. Pancreatic lipases release 2-monoacyl-sn-glycerols and free fatty acids from dietary lipids, which are used by enterocytes and acylated by acyl-coenzyme A: monoacylglycerol acyltransferase (MOGAT) to generate sn-1,2-diacylglycerols. It is worth mentioning that, in rat liver and in the small intestine, TAGs have also been reported to be synthesized in an acyl-CoA-independent manner by the actions of transacylase enzymes. In this process, a fatty acyl chain is transferred from one diacylglycerol molecule to another (Yamashita et al., 2014).

Sphingolipids are also synthesized through acylation, which involves adding fatty acids to the sphingoid backbone. The sphingoid base is formed by the condensation of an amino acid with a fatty acid. In the canonical sphingolipids, palmitoyl-CoA and the amino acid serine will generate a C18 sphingoid base (d18:n, n=0,1). This reaction is catalyzed by the enzyme serine-palmitoyl transferase, which utilizes pyridoxal 5’-phosphate(2-) as a cofactor. The amino acid moiety makes up the polar head of the sphingoid backbone, where N-acylation of the amino group generates ceramides. Different modifications of the hydroxyl group of the hydroxymethyl -R group of serine will generate sphingomyelins, glycosphingolipids, phosphate forms, and 1-O-acylceramides. It's worth noting that other combinations of amino acids and fatty acids have been reported. Originally referred to as atypical sphingolipids (Martinez et al., 2012), the incorporation of glycine or alanine instead of serine in the sphingoid backbone has significant implications. Alanine and glycine have reduced R groups, lacking the hydroxyl and methyl hydroxyl groups, respectively. Therefore, they cannot form the mentioned sphingolipids, resulting in sphingoids of (m18:n) and (m17:n) structures. Additionally, apart from palmitoyl-CoA, other fatty acids have been reported for use in forming the sphingoid backbone, including C14 or C18 fatty acids. The SPT enzyme is a heterodimer formed by SPTLC1 with either SPTLC2 or SPTLC3. An additional subunit, SPTSSA/B, binds to the heterodimer to determine substrate specificity. Various combinations of SPTLCs and SPTSSs display preferences for different amino acids and fatty acids, as reviewed by Lone MA et al. (Lone et al., 2022).
The initial acylation of the sphingoid base occurs on the amine group, leading to N-acylation, rather than O-acylation, resulting in the formation of (dihydro-) ceramides (Mullen et al., 2012). This reaction is catalyzed by ceramide synthases (CerS), a family with six members, CerS1-6. All CerSs are localized in the endoplasmic reticulum (ER), nuclear envelope, mitochondria, and mitochondria-associated membranes. Several studies have demonstrated that different CerS exhibit distinct specificity in N-acylating fatty acid-CoA based on the length of the fatty acid chain (Mullen et al., 2012). Depending on the study, slightly different combinations of fatty acid length and CerS have been reported. For example, different results can be found if the assignment was based on overexpression of the CerS or their genetic suppression. This is reviewed in Mullen et al. (Mullen et al., 2012) Levy M (Levy and Futerman, 2010).

Ceramide can additionally be acylated in position C1. It is not known what enzymes catalyze this reaction, but several enzymes have been proposed (Hernandez-Corbacho and Obeid, 2019; Rabionet et al., 2013). Nevertheless, as mentioned earlier, DGAT2 has been suggested by several authors to catalyze this reaction (Hernandez-Corbacho and Obeid, 2019). If this hypothesis holds true, DGAT2 would serve a dual function, working on both DAG and ceramide. The implications of such a scenario remain unclear. DGAT2 and other candidate enzymes are further discussed in section 4.

3. Pharmacological targeting of lipid acyltransferases

Many of the efforts in developing drugs that target glycerolipid acyltransferases are focused on diseases related to lipid accumulation. However, current strategies approved by the US Food and Drug Administration to reduce obesity and type 2 involve regulating hunger through the use of molecules that mimic the hormone glucagon-like peptide 1 (GLP-1). Although these strategies prove effective for many patients, many others do not respond well to them and experience adverse effects, including loss of muscle mass, cardiovascular disease, and osteoporosis (Lenharo, 2023). Another approach involves reducing fat intake by targeting lipid metabolism. Orlistat is a lipase inhibitor offered to reduce obesity by lowering intestinal fat absorption, although its effects on obesity are modest. Targeting the different enzymes in intestinal TAG synthesis (primarily DGAT1, but also MGATs and GPATs) has shown to be an efficient way to reduce TAGs, often leading to weight loss and improved glucose metabolism, as evidenced in animal models of obesity and human studies.

Significant interest has been focused on the use of acyltransferase inhibitors in cancer research. Current models propose communication between cancer cells and adipocytes, with the latter providing metabolic substrates to feed cancer cells (Hoy et al., 2017). The accumulation of TAGs in the form of a lipid droplet phenotype outside of adipocytes, liver cells, and macrophages is linked to metabolic diseases. And it is described in different cancers, including adenocarcinomas in the colon, prostate, pancreas, breast, and kidney (Geng and Guo, 2017). While the role of lipid droplets in cancer is not fully understood, the accumulation of lipid droplets in cancer cells has been linked to tumor proliferation, increased cancer
aggressiveness, and the invasiveness of cancer stem cells. In addition, targeting enzymes involved in TAG synthesis has demonstrated therapeutic potential in glioblastoma and other cancers (Geng and Guo, 2017). Lipid droplets have also been associated with resistance to radiotherapy and chemotherapy. For example, genetic depletion of DGAT1 was demonstrated to suppress radiotherapy resistance in glioblastoma (Kang et al., 2023).

Virtually all the enzymes in the sphingolipid pathway are being targeted in their role in many diseases, especially studied in cancer (Shaw et al., 2018). Additional pathological implications include inflammation, diabetes, cardiovascular disease, and neurodegeneration (Green et al., 2021; Hannun and Obeid, 2018). Specifically, the acylation of sphingosine to generate ceramide by CerS has been implicated in many cellular functions, such as cell death, EMT, migration, insulin resistance, and mitophagy, all of which have significant implications in cancer (Hannun and Obeid, 2018). Moreover, ceramide and other sphingolipid species have been shown to directly influence TAG formation (Ordóñez et al., 2017). However, the need for pharmacological inhibitors of CerS that are readily usable in animals and humans poses a challenge to a systemic therapeutic approach to the disease.

A new turn in ceramide signaling was recently proposed to discover that cancer cells could derivate ceramide to 1-O-acylceramide (Senkal et al., 2017). Previously, 1-O-acylceramide was primarily known for its role in protecting the skin from water loss (Rabionet et al., 2013). DGAT enzymes, mainly DGAT2, were proposed as the putative acyltransferase to catalyze this reaction. DGAT2 has also been shown to sensitize cells to radiotherapy (Nistico et al., 2021), whereas radiation-induced cell death mechanism has been reported by several works to be mediated by ceramide (Sharma and Czarnota, 2019). Therefore, the intriguing possibility that DGAT2 could play a dual role in apoptotic ceramide and TAG/Lipid droplet modulation opens a window to be explored.

Table 1 compiles the mentioned lipid acyltransferases, organized by their structural family of proteins as per the InterPro database. DGAT1 and DGAT2 share the same substrate and product (DAG to TAG); however, they belong to entirely different structural family members.

### 3.1. DGAT1

DGAT1 belongs to the MBOAT structural family of proteins, where MBOAT stands for Membrane-bound O-acyl transferase. It is classified by Pfam with the id PF03062, cluster id: CL0517, and InterPro id IPR004299. This family comprises membrane proteins that include a variety of acyltransferase enzymes responsible for transferring acyl-CoA to either a lipid molecule or a protein (Sharma and Czarnota, 2019). The sequence identity among MBOAT family members is low. However, they conserve a 3D-folding known as the MBOAT fold (Sharma and Czarnota, 2019).
DGAT1 catalyzes the final step in triacylglycerol synthesis by using diacylglycerol and fatty acyl CoA as substrates. However, less explored substrates of DGAT1 have been reported, such as retinyl esters and waxes. Thus, DGAT1 acyl-CoA: retinol acyltransferase (ARAT) activity was shown in DGAT1-overexpressing insect cells and in murine and mammalian cells (Batten et al., 2004; Yen et al., 2005). DGAT1 is the major ARAT enzyme in the skin, preventing retinoid toxicity. Additionally, it also possesses acyl-CoA:monoacylglycerol acyltransferase (MGAT) activity, suggesting that the DGAT1 could catalyze a two-step reaction from MAG to TAG (Yen et al., 2005).

The primary focus of interest in inhibiting DGAT1 has been the prevention of fat absorption following food intake. In a study published in 2000 by Farese’s laboratory, conducted prior to the identification of Dgat2, mice lacking Dgat1 were found to be resistant to high-fat diet-induced obesity (Smith et al., 2000). These mice exhibited reduced white adipose tissue, decreased liver TAG levels, while maintaining normal plasma TAG levels. Additionally, lactating female mice were unable to produce milk due to the absence of lipid droplet accumulation in the mammary tissue. Two years later, findings from the same laboratory revealed that the reduced TAG levels in various tissues (such as skeletal muscle, liver, and white adipose tissue), along with smaller adipocytes, led to improved sensitivity to insulin and leptin when mice were fed in a high-fat diet (Chen et al., 2002a). Furthermore, the reintroduction of intestinal Dgat1 in knockout (KO) mice reversed the phenotype to that of the wild type (Hung et al., 2017). These findings motivated several lines of investigation in developing DGAT1 inhibitors to fight obesity and type 2 diabetes. Most representative or used DGAT1 inhibitors are summarized:

The DGAT1 inhibitor T863 was patented by the companies Tularik Inc. and Japan Tobacco Inc. in 2004. In addition to initial studies demonstrating its effects on DGAT1 and its ability to reduce TAG content, this drug underwent further testing to assess its selectivity and potency against DGAT1 (Cao et al., 2011). T863 binds to fatty acyl-CoA substrate binding tunnel, which is consistent with previous reports suggesting that T863 is competes with the fatty acid-CoA for enzyme binding. Furthermore, DGAT1 inhibitor T863 does not show inhibition of other tested acyltransferases such as MOGT3, MOGT2, and DGAT2, and is minimally active against another MBOAT member, SOAT-1 (Sui et al., 2023). Cell studies showed inhibition of TAG formation but an increase in phospholipid species upon T863 treatment. Animal studies in mice fed a high-fat diet also revealed a decrease in serum TAG levels and a delay in the accumulation of lipid droplets in enterocytes in mice treated with T863. A two-week treatment period led to weight loss in mice and reduced serum TAGs and cholesterol levels, along with improved glucose tolerance and insulin sensitivity (Cao et al., 2011). The crystal structure of DGAT1 with T863 was resolved in 2023 (Sui et al., 2023), confirming that the inhibitor binds the fatty acid-CoA binding tunnel, thereby obstructing the entry of fatty acids (Sui et al., 2023).

PF-04620110 was developed by Pfizer Global Research and Development (Dow et al., 2011b). In vitro microsomal assays, PF-04620110 exhibited selectivity for DGAT1 (IC50 19nM) when compared to DGAT2, AWAT1/2, and MOGAT2/3 (Enayetallah et al., 2011). In mice, this compound (0.1 mg/kg) lowered serum
TAG levels and mitigated high-fat diet-induced chronic inflammation in the type 2 diabetes mouse model (Jo et al., 2019). Modification of the chemical structure by incorporation of a dioxino[2,3-d]pyrimidine-based core to this inhibitor, reduced polarity (initial value log D7.4 = −0.15), and enhanced passive permeability (Dow et al., 2011a). Furthermore, when combined with a DGAT2 inhibitor (PF-06424439), it reduced TAG secretion and accumulation in the blood and intestine in high-fat diet mouse models. However, it increased fecal fatty acid excretion, leading to severe diarrhea and death. None of the inhibitors alone had these effects (Takemoto et al., 2020), due to the compensatory role of DGAT2 when DGAT1 is inhibited.

Compound AZD7687, developed by AstraZeneca R&D was based on a pyrazinecarboxamide core structure, and it is another inhibitor reported to be selective and potent (EC50 11nM) in inhibiting DGAT1, in comparison to DGAT2 and SOAT1/2. AZD7687 entered multiple phase 1 clinical trials for the treatment of type 1 diabetes and obesity. While oral administration of AZD7687 reduced postprandial TAG blood levels, higher doses led to nausea, vomiting, and diarrhea in healthy subjects (Denison et al., 2013; Denison et al., 2014). Benzimidazole-based DGAT1 inhibitors have shown similar phenotypes (Serrano-Wu et al., 2012).

A compound based on piperidinyl-oxycyclohexanecarboxylic acid reported an IC50 of 9nM for human DGAT1 compared to 10uM for SOAT1. It also demonstrated improved off-target effects against the adenosine A2A receptor, an off-target identified in initial studies (He et al., 2014) (2014). Pradigastat (LCQ-908, Novartis AG), DGAT1 inhibitor (Bauer et al., 2016), went to phase 2 clinical trial, demonstrating to be well-tolerated in patients and to be able to decrease TAGs at a daily dose of 20mg (Meyers et al., 2015), and proposed to treat familial chylomicronemia syndrome (Meyers et al., 2015). A-922500 (Abbott Laboratories, 2009) was tested in diet-induced rat models of hypertriglyceridemia at 3 mg/kg, reducing circulating TAGs and free fatty acids. This compound was also used to block lipid droplet formation in response to mustard gas in cell cultured human bronchial epithelial cells (Ye et al., 2023).

GSK3008356, developed by GlaxoSmithKline and featuring an unrelated chemical structure, is a selective DGAT1 inhibitor developed to address non-alcoholic steatohepatitis (NASH). As with the other DGAT1 inhibitors, it also decreased circulating TAGs (Okour et al., 2019).

It is also important to mention that DGAT1 KO mice suffer from skin defects, such as hair loss and sebaceous gland atrophy, defects in protecting from water loss, and thermoregulation (Chen et al., 2002b). Mice treated with the DGAT1 inhibitor AZD7687 exhibited similar skin and hair defects. When tested in dogs, AZD7687 was shown to affect skin hair (Floettmann et al., 2015), suggesting possible long-term side effects on humans. Likewise, compound LCQ-908 showed mild local skin erythema reactions upon exposure to light. Subsequent studies determined these compounds to be safe at patient doses (Bauer et al., 2016). Compound A-922500 was reported to primarily distribute in the intestine without producing skin aberrations in the tested animals (Bauer et al., 2016).
The results from clinical studies revealed the limitations of targeting DGAT1 in humans. While mice treated with DGAT1 inhibitors (or Dgat1 knockout) remained healthy, multiple gastrointestinal side effects were observed in human subjects. Interestingly, DGAT1 mutations were identified in patients with congenital diarrheal disorders (Gluchowski et al., 2017; Haas et al., 2012; van Rijn et al., 2018). However, only mice co-treated with DGAT1 and DGAT2 inhibitors exhibited side effects. Thus, the lower expression of DGAT2 in the human intestine may explain the discrepancies observed between mice and human models when using DGAT1 inhibitors. A better understanding of the mechanisms by which DGAT1 inhibition leads to gastrointestinal adverse events and how they can be mitigated will be crucial for the treatment of diabetes and obesity by targeting DGAT1.

3.2. DGAT2

DGAT2 and MGATs acyltransferases belong to the structural DAGAT family, which stands for Diacylglycerol acyltransferase (PF03982, integrated to IPR007130, with Clan CL0228). In humans, this family contains 7 members. They are responsible for synthesizing TAGs, wax esters, and DAG from dietary 2-monoacylglycerols. Within this family, Acyl-CoA wax alcohol acyltransferase 1 (AWAT1, Q58HT5) and 2 (AWAT2/ MFAT, Q6E213) catalyze the formation of ester bonds between fatty alcohols and fatty acyl-CoAs to form wax monoesters, which are important for skin integrity. AWAT2, also known as multifunctional O-acyltransferase (MFAT), exhibits acyl-CoA retinol acyltransferase (ARAT) activity, specifically catalyzing the synthesis of 11-cis-specific retinyl esters. ARAT2 displays higher catalytic efficiency with 11-cis-retinol compared to 9-cis-retinol, 13-cis-retinol, and all-trans-retinol substrates. This enzymatic activity has been shown to play a role in the cone-specific retinoid (visual) cycle, which is essential for color vision.

DGAT2 catalyzes the last step in the TAG pathway, and it was identified to address the DGAT activity in DGAT1-deficient (Dgat1−/−) mice. While these mice exhibited reduced body mass, their plasma TAG levels remained normal (Smith et al., 2000). Two distinct peptides with DGAT activity were discovered in the soil fungus Mortierella ramanniana (Lardizabal et al., 2001), which eventually led to the identification of DGAT2 in humans (Cases et al., 2001). DGAT2 exhibits a distinct distribution compared to DGAT1 and is primarily expressed in fatty tissues such as adipose, liver, and mammary gland (Hernandez-Corbacho and Obeid, 2019). Like DGAT1, DGAT2 is also found in the ER. However, DGAT2 is also present and associated to lipid droplets (Senkal et al., 2017) and, under certain conditions, co-localize with the mitochondria-associated membranes (MAMs)(Stone et al., 2009).

In contrast to DGAT1, DGAT2 is essential for survival. Mice deficient for DGAT2 die in the early postnatal period due to abnormalities in energy and skin homeostasis (Stone et al., 2004). However, the use of tissue-specific antisense oligonucleotides targeting DGAT2 in animals has enabled the identification of specific effects. Therefore, suppressing DGAT2 expression, but not DGAT1, reduced TAG levels in the liver and improved hepatic insulin sensitivity in a diet-induced hepatic steatosis animal model (Choi et al., 2007). Knockdown of DGAT2 in the liver showed reduced TAG synthesis, VLDL TAG, Apo B secretion and plasma...
cholesterol as compared to wild-type mice (Liu et al., 2008). Knockdown of DGAT2 in liver and adipose tissue, reduced hepatic lipogenesis, hepatic steatosis, and attenuated hyperlipidemia in both high-fat diet-induced obese and ob/ob mouse models (Yu et al., 2005).

Moreover, several works have reported that DGAT2 modulates the ceramide metabolism. Overexpression of DGAT2 in muscle resulted in the accumulation of ceramide (Watt, 2009), whereas overexpression of DGAT1 has been found to reduce ceramide in cardiac muscle (Liu et al., 2009). As will be discussed in section 4 in detail, DGAT2 has been reported to have ceramide acyltransferase activity and sensitizes cells to apoptotic cell death (Hernandez-Corbach and Obeid, 2019).

**DGAT2 inhibitors**

Treatment with the DGAT2 inhibitor **PF-06427878** led to a decrease in hepatic and circulating plasma TAG concentrations and reduced lipogenic gene expression in rats on a Western-type diet (Amin et al., 2019). In a mouse model of non-alcoholic steatohepatitis (NASH), PF-06427878 also ameliorated steatosis, ballooning, and fibrosis in the liver without adverse effects (Lardizabal et al., 2001). These promising outcomes were extended to humans in two phase 1 clinical trials. PF-06427878 was administered for 14 days with repeated dosing (15 to 1500 mg/day). The inhibitor was well received even at the highest dose, with only occasional side effects noted, including diarrhea, abdominal pain, and headache. Liver function indicators (Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) markers), showed improvement in healthy adults at the highest doses compared to the placebo. Elevated ALT and AST are considered a hallmark of nonalcoholic fatty liver disease (NAFLD). Liver fat was also reduced, though not plasma TAGs. The clinical trials were discontinued due to constraints in size, chemical properties of the DGAT2 inhibitor and the lack of significance effects at dose lower than 1500 mg/day (Lardizabal et al., 2001).

The promising therapeutic potential of PF-06427878 resulted in the identification of a more selective and potent compound, PF-06865571 (**ervogastat**) (Futatsugi et al., 2022). Ervogastat is currently in phase 2 clinical trials for the treatment of non-alcoholic steatohepatitis (NASH) with liver fibrosis (Futatsugi et al., 2022).

The DGAT2 inhibitor PF-06424439 reduces circulating and hepatic lipids when given orally to dyslipidemic rodent models (Futatsugi et al., 2022). This compound has also been employed in breast and colon cancer studies. In breast cancer, it was demonstrated to diminish lipid droplet formation, increase cell sensitivity to nutritional stress (Almanza et al., 2022) and heighten cell responsiveness to radiation (Nistico et al., 2021). In colon cancer, it was revealed to inhibit acylceramide formation, providing evidence for DGAT2 as a ceramide acyltransferase (CAT) enzyme (Senkal et al., 2017).

**3.3 GPATs**
GPAT enzymes catalyze the acylation of glycerol-3-phosphate to form mono-acylglycerol-phosphate, the precursor of lysophosphoanolipids, phospholipids, DAG, and TAGs. GPAT1 and GPAT2 belong to the GPAT/DHAPAT (Interpro F-IPR022284) family, whereas GPAT3 and GPAT4 do not belong to a specific family but share the structural LPCAT1-like domain (InterPro D-IPR045252) (Yu et al., 2018).

In addition to structural differences, GPAT1 and 2 are found in the MAMs, while GPAT3 and 4 are situated in the ER. GPAT1 was discovered to have high expression in AML cells, and silencing it suppressed the cell growth of AML (Irifune, 2022). GPAT1 is the primary contributor to TAG synthesis in adipocytes and hepatocytes, accounting for over half of the total GPAT activity in rodent liver. GPAT2 is found in the testis and was initially cloned from there (Wang et al., 2007). GPAT2 is also detected in tumor tissues and plays a role in tumorigenesis in various cancer types (Fratta et al., 2011). Overexpression of GPAT2 indeed stimulated cell proliferation and migration in breast cancer cell lines, such as MDA-MB-231 cells. GPAT2 is also involved in the biosynthesis of the small non-coding Piwi-interacting RNA (piRNA) (Pellon-Maison et al., 2014).

**GPAT inhibitors**

GPAT1 is the only GPAT enzyme resistant to inactivation induced by sulfhydryl-group modifying reagents like N-ethylmaleimide (NEM) (Yu et al., 2018). N-ethylmaleimide is a small molecule that forms covalent thioether bonds with cysteines, preventing disulfide bond formation. NEM is typically employed to differentiate GPAT1 activity from that of the other forms (GPAT2-4) (Gonzalez-Baro et al., 2007).

In a search for compounds to reduce body fat, GPAT activity was the focus, leading to the design and synthesis of several benzoic and phosphonic acids (Wydysh et al., 2009). Among them, FSG67, 2-(nonylsulfonamido) benzoic acid, displayed the most potent inhibitory activity (IC50 = 24.7 μM) (Wydysh et al., 2009). Additionally, FSG67 was discovered to inhibit GPAT3 expression (at 150μM), leading to reduced pro-inflammatory cytokine secretion in Kupffer cells (Fan et al., 2023). Furthermore, GPAT3 knockout mice exhibited decreased plasma inflammatory markers and reduced Kupffer cell responses, as well as smaller liver lipid droplets compared to wild-type mice under LPS stimulation (Fan et al., 2023). In a different study, FSG67 inhibitor was used to block PA synthesis to reveal the regulation of GSK3 by PA (Clemens et al., 2019). *In vivo* experiments showed body weight loss in diet-induced obese mice with a daily administration of FSG67 (5 mg/kg, 15.3 μmol/kg)(Kuhajda et al., 2011). In pancreatic adenocarcinoma cells, this inhibitor decreased cellular lipid content and increased sensitivity to several chemotherapeutical compounds (Kaoutari et al., 2021) and reduced LPA signaling in acute myeloid leukemia (Irifune et al., 2023).

### 3.4 AGPAT
AGPATs catalyze the second acylation step in the glycerol phosphate pathway, which converts lysophosphatidate to phosphatidate by adding an acyl group to the sn-2 position of the glycerol backbone. AGPAT1 and AGPAT2, both enzymes localized in the ER, are the most extensively studied AGPATs. While other AGPATs have been identified based on sequence similarity, their in vivo functions remain relatively unexplored (Takeuchi and Reue, 2009). AGPAT 1 to 5 are structure related and belong to PL-GAT domain (D-IPR002123). Overexpression of AGPAT1 stimulates TAG formation, glucose uptake and its incorporation into lipids in adipose tissue and skeletal muscle (Ruan and Pownall, 2001). Mutation of AGPAT2 in humans results in the inhibition of triacylglycerol synthesis and storage in adipocytes, and it has been related to congenital generalized lipodystrophy, with reduced adipose tissue, insulin resistance, hypertriglyceridemia, hepatic steatosis and diabetes (Agarwal et al., 2002). Intriguingly, Agpat2-/- mice, mirroring the phenotype seen in humans with AGPAT2 mutations, exhibited a significant increase in the expression of sphingolipid synthetic enzymes. This resulted in elevated levels of sphingolipid species in the liver, including ceramide and sphingosine-1-phosphate, highlighting connections between glycerolipids and sphingolipids pathways (Sankella et al., 2017).

**AGPAT inhibitors**

In a study investigating the proteasome inhibitor PS-341 (Bortezomib, Velcade), a decrease in the expression of lipogenic genes, including AGPAT and DGAT under the control of SREBP-1c, was observed. The authors proposed the potential use of this compound in alcohol-induced liver steatosis. Ethanol consumption was shown to upregulate the expression of AGPAT and DGAT enzymes, leading to the accumulation of TAGs and other lipids. However, co-treatment of rats with ethanol and the inhibitor PS-341 prevented the ethanol-induced increase in lipid synthesis (Oliva et al., 2012). PS-341 has also been demonstrated to induce ceramide production, resulting in apoptosis in pancreatic cell lines, and it may play a role in regulating CerS2 and CerS6 (Lee et al., 2022). Whether the effects on AGPAT and ceramide production were interrelated was not investigated (Gong et al., 2014).

### 3.5 Ceramide Synthases

Ceramide synthases (CerS) are members of the Lag1/Lac1-like family (InterPro F-IPR016439) and play a crucial role in sphingolipid synthesis, a topic extensively reviewed by Hannun YA (Hannun and Obeid, 2008). CerS enzymes N-acylate dihydrosphingosine to dihydroceramide, which can then be further reduced to ceramide. Alternatively, CerS can directly generate ceramide through N-acylation of sphingosine. Ceramide is regarded as the central molecule in sphingolipid signaling and metabolism, with the complexity of ceramide signaling reviewed in (Hannun and Obeid, 2011), and (Canals and Clarke, 2022). In the context of cell signaling, ceramide has been implicated in a broad range of biological functions, predominantly apoptotic cell death, cell cycle arrest, and senescence. More recently, our research group has shown how plasma membrane ceramide regulates cell adhesion and cell migration in response to stimulation (Canals et al., 2020; Greene et al., 2023). Ceramide is the precursor of sphingolipid metabolism, generating
sphingomyelins, hexosylceramides, complex glycosphingolipids, 1-phosphate forms, and acylceramides. Its catabolism also generates bioactive molecules such as sphingosine and sphingosine-1-phosphate. Due to their role in many biological functions, the sphingolipid metabolizing enzymes have been the focus of extensive drug discovery efforts (Canals and Hannun, 2013; Canals et al., 2011).

There are six different CerS enzymes, numbered from 1 to 6. Each CerS exhibits specificity for various fatty acid-CoA substrates, leading to the formation of distinct (dihydro)ceramide species. As mentioned before, there is not a unanimous agreement regarding the specific fatty acid-CoA preferences for each CerS, mainly because they have been evaluated in different experimental systems and with diverse fatty acid/CerS combinations. Nevertheless, there is consensus that CerS1 have preference for C18, and C18:1-acyl-CoAs; CerS2 for C20:0, C22:0, C24:0, C24:1, and C26:0-acyl-CoAs; CerS3 for C18:0-to C26:0-acyl-CoAs, and even longer ultra-long acyl-CoAs; CerS4 for C18:0 and C20:0-acyl-CoAs; CerS5 and CerS6 for C14:0 and C16:0-acyl-CoAs (Levy and Futerman, 2010; Mullen et al., 2012).

Although all CerS enzymes are primarily localized in the endoplasmic reticulum (ER), some studies have reported their presence in the Golgi and nuclear membranes. Based on mRNA expression, CerS1 is predominantly found in the brain and testis (Brachtendorf et al., 2019). CerS2, on the other hand, exhibits ubiquitous expression and abundance in all tissues, with notably high protein levels in the lung, intestines, kidneys, liver, and spleen (Schiffrmann et al., 2013). While CerS3 is primarily specific to the testis, it plays crucial roles in skin tissue, making it essential for life. CerS4 is detected in various tissues, including the skin, heart, brain, leukocytes, and liver. CerS5 is highly expressed in lung epithelia but is broadly present in many tissues, including the testis, kidney, lung, skeletal muscle, heart, and brain. CerS6 is predominantly expressed in the intestine, kidney, liver, and brain (Schiffrmann et al., 2013).

CerS enzymes have been reported to play a critical role in mediating various ceramide-induced biological processes. For example, the loss of CerS1 and ceramide (d18:1/18) in HNSCC tumors is associated with lymphovascular invasion and nodal metastasis (Karahatay et al., 2007). In A549 human lung adenocarcinoma cells, ceramide generated from CerS1 was found to repress the promoter activity of human telomerase reverse transcriptase (hTERT) (Wooten-Blanks et al., 2007). Conversely, elevated CerS1 levels in breast cancer and colon cancer are correlated with poor prognosis. Mice deficient for CerS2 experience a depletion of very long-chain ceramides (d18:1/24:0, d18:1/24:1) and develop liver adenoma and hepatocellular carcinoma (Imgrund et al., 2009). CerS4 exhibits high expression levels in several cancers. Analysis of cancer tissues from patients has revealed elevated levels of CerS5, both in mRNA expression and protein levels, in many cancers, such as colorectal and gastric cancer (Fitzgerald et al., 2015; Zhang et al., 2022). CerS5 is reported as a marker in colorectal canc er (Kijanka et al., 2010). Interestingly, CerS6 has been identified as a target of the p53 protein, exhibiting a contrasting role in cancer progression (Fekry et al., 2016a; Fekry et al., 2016b). Mechanistically, CerS5 has been implicated in triggering apoptotic cell death in response to various stimuli. For instance, CerS6, in conjunction with
CerS5, is involved in programmed cell death mechanisms downstream of cytotoxic compounds, including UV light, and is necessary for the generation of apoptotic ceramide leading to cell death. However, in MCF-7 cells, TNF-alpha requires CerS6 but not CerS5 to induce cell death, indicating that they do not have redundant functions (Hernandez-Corbacho et al., 2015).

CerS inhibitors

The study of CerS enzymes is fundamentally associated with two inhibitors: myriocin and fumonisin B1 (FB1). Myriocin serves as an inhibitor of Serine Palmitoyl Transferase (SPT), the initial enzyme in the sphingolipid pathway, while FB1 inhibits all CerS isoforms. Since both myriocin and FB1 act on the de novo pathway of sphingolipids, any effects that are blocked by FB1 but not by myriocin are attributed to CerS (Hernandez-Corbacho et al., 2015).

An analogue of myriocin and sphingosine, known as FTY720 (or fingolimod), does not inhibit the SPT enzyme. FTY720 can function as a sphingosine-1-phosphate receptor agonist and is utilized for the treatment of multiple sclerosis (Chung et al., 2023). When it targets S1PR1 in lymphocytes, it leads to their internalization and degradation, making it an immunosuppressive agent. Intriguingly, FTY720 has also been reported to have additional molecular targets. Specifically, it has been identified as targeting the PP2A inhibitor SET, thereby activating PP2A, and it has been described to have inhibitory effects on CerS (Vicente et al., 2020). AAL(S) an analogue of FTY720, cannot be phosphorylated by sphingosine kinase 2 and does not act as an S1PR antagonist. Nevertheless, AAL(S) maintains its inhibitory effects on CerS, with a greater specificity toward CerS1 (Toop et al., 2015).

The first isoform-specific ceramide synthase inhibitor, P053, has an IC50 of 0.5 μM, displaying potent inhibition of CerS1. Chronic administration of P053 led to increased fatty acid oxidation and a blockade in the rise of TAG production in muscle and adipocytes within an animal model of high-fat diet-induced metabolic disease. Notably, P053 had no impact on insulin resistance. The IC50 values for the inhibition of CerS2, CerS4, CerS5, and CerS6 were at least one order of magnitude higher than that for CerS1, illustrating a strong selectivity for CerS1 over the other CerS isoforms. The IC50 for CerS1 was similar to the potency of FB1. In cultured cells, when used at 300 nM, P053 reduced levels of C18 ceramides, sphingomyelins, and hexosylceramides (Turner et al., 2018).

4. New lipid in the Signaling arena: 1-O-acylceramide

In 1983, O-acylceramides were identified as skin components using rats as animal models (Wertz et al., 1983). These O-acylceramides primarily consist of linolenic ω-linked to 30- to 34-carbon alkyl groups of ceramide and phytoceramide (ω-hydroxy acids in amide linkage with sphingosine). The function of ω-O-acylceramides was found to be the prevention of water loss through the epidermis, which is crucial for maintaining life (Ponec et al., 2003). It was suggested that the synthesis of ω-O-acylceramides occurs
through hydroxylation (via cytochrome P450) and acylation of glucosylceramide, followed by
deglycosylation catalyzed by beta-glucocerebrosidase (Takagi et al., 2004). Defects in TAG metabolism in
the skin, such as those in humans with mutations in the ABHD5 gene, result in the inability to provide fatty
acids for ω-O-acylceramide formation (Radner and Fischer, 2014). Similarly, mutations in CerS3 lead to the
failure to generate the ceramide moiety in the skin (Jennemann et al., 2012). As a result, ω-O-acylceramides,
consisting of ultra-long ceramides, constitute the extracellular lipid lamella in the stratum
corneum of the epidermis. Subsequently, another family of O-acylceramides was identified as a natural
component of human and mouse epidermis, where the additional acyl group is not linked to the omega
position but to position 1 of ceramide, forming 1-O-acylceramides (Figure 2) (Lin et al., 2017; Rabionet et
al., 2013).

First reported acylceramide synthase (ACS): LPLA2, lysosomal phospholipase A2 group XV.

The first enzyme identified to catalyze the synthesis of 1-O-acylceramides was discovered during a
pharmacological study of novel glucosylceramide synthase inhibitors. Glucosylceramide synthase (GCS)
catalyzes the formation of glucosylceramide from the substrate ceramide and UDP-glucose. The inhibitor D-
threo-1-Phenyl-2-decanoylamino-3-morpholino-propanol (PDMP) suppresses GCS activity, leading to a
reduction in glucosylceramide and complex glycosphingolipid synthesis (Shayman et al., 2004). Initially, the
accumulation of cellular ceramide upon treatment with PDMP was interpreted as an accumulation of the
substrate of the reaction. However, the development of more potent PDMP analogs revealed that a higher
percentage of GCS inhibition did not result in a higher ceramide accumulation (Lee et al., 1999). Moreover,
an enantiomer that was inactive towards GCS still led to the accumulation of ceramide, suggesting the
presence of other targets in ceramide metabolism. None of the known enzymes capable of metabolizing
ceramide showed inhibition, including sphingomyelin synthase, acid and alkaline ceramidase, acid and
neutral sphingomyelinase, and ceramide synthases. The source of ceramide was traced using radiolabeled
probes, leading to the conclusion that PDMP treatment inhibited the formation of acylceramides (Lee et al.,
1999). This reaction was inhibited by the addition of fatty acids but enhanced by the addition of liposomes
containing phospholipids, suggesting that acylation of ceramide occurred through transacylation with a
phospholipid as the substrate. The authors proposed an enzymatic mechanism in which the sn-2-acyl group
of phosphatidylethanolamine or phosphatidylcholine was transferred to the 1-hydroxyl group of ceramide.
This enzyme was identified as a calcium-independent phospholipase A2 (PLA2), a heterogeneous group of
enzymes that hydrolyze the ester fatty acid at position sn-2 of glycerophospholipids, releasing a free fatty
acid and a phospholipid. In addition to water, this enzyme was found to transacylate other substrates, such
as sphingosine (Peng et al., 2021). The purification of the enzyme from rat brain revealed its acidic pH
optima, significant mannose content, and localization in lysosomes (Abe et al., 1996). Immunoprecipitation
of overexpressed tagged-PLA2 confirmed its 1-O-acyltransferase activity (Hiraoka et al., 2002). A
comparison with the human genome led to the identification of an enzyme similar to lysophospholipase,
known as LCAT-like lysophospholipase or LLPL (Shayman et al., 2004). The current recommended name is phospholipase A2 group XV (PLA2G15, Uniprot: Q8NCC3, PAG15_HUMAN).

**Putative role of DGAT2**

In yeast, 1-O-acylceramide synthesis was reported by two enzymes: phospholipid diacylglycerol acyltransferase (Lro1p) and diacylglycerol O-acyltransferase (Dga1p). Accordingly to the database *Alliance of Genome Resources* (https://www.alliancegenome.org), Lro1p is orthologous to both the human LCAT (lecithin-cholesterol acyltransferase) and PLA2G15. Dga1p is orthologous to several human genes in the DAGAT structural family of proteins, including MOGATs and DGAT2 (Hernandez-Corbacho and Obeid, 2019).

Synthesis of 1-O-acylceramide via DGAT2 has been suggested to occur in lipid droplets, rather than in the ER (Voynova et al., 2012). Microsomal preparations of Dga1p overexpression failed to acylate ceramides with different acyl-CoAs *in vitro*. However, the Dga1p-dependent synthesis of O-acylceramides was proven in labeling experiments *in vivo*. Using human HCT-116 colon cancer cells, the loss of DGAT2 resulted in increased ceramide levels and decreased O-acylceramides (Senkal et al., 2017). Conversely, overexpression of recombinant DGAT2 induced the accumulation of O-acylceramides within the cells. Moreover, *in vitro* synthesis of O-acylceramide was significantly reduced by using the DGAT2-specific inhibitor PF-06424439 in a dose-dependent fashion. The authors suggested that DGAT2, in collaboration with CerS and ACSL5, is involved in the formation of cellular O-acylceramides within the lipid droplets. Furthermore, in the same study, DGAT2 was shown to be necessary for 1-O-acylceramide synthesis in mouse liver upon a high-fat diet (Senkal et al., 2017).

**Putative role of DGAT1**

Similar to DGAT2, which catalyzes the same reaction as DAG-acyltransferase, DGAT1 has also been investigated for its potential to acylate ceramides. While not a direct activity, DGAT1 was shown to influence the levels of 1-O-acylceramide (Bayerle et al., 2020; Rabionet et al., 2022). Mice deficient in Dgat1 showed a reduction in 1-O-acylceramide in the colon, although not in the small intestine or liver (Bayerle et al., 2020). Additionally, microsomes of cultured cells overexpressing murine Dgat1 or Dgat2 were capable of forming acylceramide *in vitro*, compared to non-transfected control microsomes (Senkal et al., 2017).

**Other studies**

The elusive identification of the enzyme responsible for the biosynthesis of 1-O-acylceramides led to a recent study to analyze the several enzymes implicated in this product. Mice mutants for Cers2, Cers3, Cers4, DGAT1, DGAT2, Elovl3, LCAT, Scd1 and ACDase were analyzed. However, none of them showed a decrease in 1-O-acylceramides. Nevertheless, the DGAT2 mutant reshaped acylceramide composition, suggesting that it was somewhat related. These works concluded that some of these enzymes might have redundant functions, or the enzyme responsible has not been discovered yet (Rabionet et al., 2022).
5. Conclusions

Fatty acids are the building blocks of structural and signaling lipids, and adding these discrete units by lipid acyltransferases allows the building of a complex network of structures. The specificity of acyltransferases to particular reactions has allowed the development of potent inhibitors. Much of the effort in drug development in glycerolipids metabolism has focused on metabolic diseases related to TAG accumulation. In contrast, the main inhibitors targeting the acylation of sphingolipids have focused on cancer. Glycerolipids and sphingolipids biosynthesis starts from a very distinct substrate, but they follow analog sequential acylations. The biophysics similarities between the structures after each acylation cycle make them share signaling and biological functions. However, few works have tried to understand how these two worlds interact.

Notably, 1-O-acylceramides are the sphingolipid counterparts of TAGs in the glycerolipid family. They are even more hydrophobic than TAGs and are also present in lipid droplets, where they protect the skin from water loss. The acyltransferase responsible for acylating ceramide to form 1-O-acylceramides remains to be discovered, although several independent studies have pointed to DGAT2. If DGAT2 is indeed the enzyme, it would have dual roles in glycerolipid and sphingolipid metabolism. If this is the case, it could imply that some biological processes, particularly in cancer, attributed to DGAT2 and TAG formation may be due to ceramide depletion and the formation of 1-O-acylceramides. If DGAT2 is not the responsible enzyme, researchers need to identify this enzyme and assess the functions of 1-O-acylceramides in cells, as well as their role in high-fat diets and cancer.

Plutarch's 'Parallel Lives,' written by the ancient Greek historian and biographer, compares the biographies of notable figures from Greek and Roman history, drawing lessons from their similarities and differences. Similarly, glycerolipids and sphingolipids exhibit a parallel relationship, with advances in one class of lipid often leading to progress in the other.
Author contributions

Wrote the manuscript: Hernandez-Corbacho, M, and Canals, D.
Footnotes

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No author has an actual or perceived conflict of interest with contents of this article.

This article contains no datasets generated or analyzed during the current study.
Figure legends

Figure 1. Lipid acyltransferase enzymes in the glycerol-phosphate and sphingolipid pathways. Acyltransferases for the glycerol phosphate and sphingolipid pathways are shown in colors. Colors designate a structural group. For clarity also SPT and sphingomyelin synthases (SMS1/2) are shown.

Figure 2. Structures of (A) 1-O-acylceramide, (B) ω-O-acylceramide, and (C) triacylglyceride. Backbone structure (sphingosine in A and B; and glycerol in C) is colored in black, N-linked acyl is in brown. Omega-fatty acid is in purple. O-acyl acid in teal. The first 5 carbons of the sphingoid base, and the 3 glycerol carbons in the triacylglyceride, are numbered to show positions in the structure.
Table 1. Lipid acyltransferases enzymes grouped by the InterPro family members or domains.

<table>
<thead>
<tr>
<th>F-Family D-Domain (InterPro/Pfam)</th>
<th>Name</th>
<th>Gene name</th>
<th>Uniprot</th>
<th>Subcellular localization</th>
<th>Reaction</th>
<th>Tissue</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lysophosphatidylcholine Acyltransferase</strong> (MBOAT)</td>
<td>LPCAT3 (MBOAT5)</td>
<td>Q6PTA2</td>
<td>endoplasmic reticulum</td>
<td>LPC → PC LPS → PC LPE → PE</td>
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<td>[Zhao et al., 2008]</td>
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<td><strong>Diacylglycerol O-Acyltransferase</strong></td>
<td>DGAT1 (ARAT)</td>
<td>Q7S807</td>
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<td>AG → TAG ATRA → FA ATRA MAG → MAC</td>
<td>intestine, liver, breast</td>
<td>[Ma et al., 2017]</td>
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<td>Cho → FA-Chol</td>
<td>adrenal</td>
<td>[Lin et al., 1999]</td>
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<td>Q6YN28</td>
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<td>not specificity</td>
<td>[Wu et al., 2008]</td>
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<td>Q6YW17</td>
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<td>LPA → PA LPE → PE LPS → PS</td>
<td>not specificity</td>
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<td>MOAT3 (MOGAT3)</td>
<td>Q5Y9F5</td>
<td>endoplasmic reticulum</td>
<td>OA → TAG</td>
<td>colon, liver</td>
<td>[Cheng et al., 2003]</td>
<td></td>
</tr>
<tr>
<td><strong>Diacylglycerol Acyltransferase</strong> (DAGAT)</td>
<td>DGAT1</td>
<td>Q8MDT8</td>
<td>endoplasmic reticulum Lipid Droplets</td>
<td>OA → TAG</td>
<td>adipose tissue, breast, liver, skin</td>
<td>[Cases et al., 2001; Wakimoto et al., 2003]</td>
<td></td>
</tr>
<tr>
<td><strong>Acyl-CoA wax-alcohol acyltransferase 1</strong></td>
<td>AWAT1</td>
<td>Q5H1T5</td>
<td>endoplasmic reticulum</td>
<td>OA-OH → Wax</td>
<td>skin, thymus, prostate and testis</td>
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<td></td>
</tr>
<tr>
<td><strong>Acyl-CoA wax-alcohol acyltransferase 2</strong></td>
<td>AWAT2</td>
<td>Q5E213</td>
<td>endoplasmic reticulum</td>
<td>OA-OH → Wax</td>
<td>skin</td>
<td>[Cheng and Russell, 2004]</td>
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</tr>
<tr>
<td><strong>2-acylgllycerol O-Acyltransferase 1</strong></td>
<td>MOAT1 (MOGAT1)</td>
<td>Q5D9P6</td>
<td>endoplasmic reticulum</td>
<td>2OA → DAG</td>
<td>stomach and liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2-acylgllycerol O-Acyltransferase 2</strong></td>
<td>MOAT2 (MOGAT2)</td>
<td>Q5SDYC2</td>
<td>endoplasmic reticulum</td>
<td>2OA → DAG</td>
<td>liver, small intestine, colon, stomach and kidney</td>
<td>[Yen and Farese, 2007]</td>
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</tr>
<tr>
<td><strong>2-acylgllycerol O-Acyltransferase 3</strong></td>
<td>MOAT3 (MOGAT3)</td>
<td>Q5Y9F5</td>
<td>endoplasmic reticulum</td>
<td>2OA → DAG</td>
<td>colon, liver</td>
<td>[Cheng et al., 2003]</td>
<td></td>
</tr>
<tr>
<td><strong>Sphingosine-1 Phosphatase Acyltransferase 3</strong></td>
<td>MOAT1</td>
<td>Q8MDT8</td>
<td>endoplasmic reticulum Lipid Droplets</td>
<td>OA → TAG</td>
<td>adipose tissue, breast, liver, skin</td>
<td>[Cases et al., 2001; Wakimoto et al., 2003]</td>
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</tr>
<tr>
<td><strong>Sphingosine-1 Phosphatase Acyltransferase 2</strong></td>
<td>MOAT2</td>
<td>Q6NUJ2</td>
<td>endoplasmic reticulum</td>
<td>OA → TAG</td>
<td>adipose tissue, breast, liver, skin</td>
<td>[Cases et al., 2001; Wakimoto et al., 2003]</td>
<td></td>
</tr>
<tr>
<td><strong>Sphingosine-1 Phosphatase Acyltransferase 2</strong></td>
<td>MOAT3 (MOGAT3)</td>
<td>Q5Y9F5</td>
<td>endoplasmic reticulum</td>
<td>OA → TAG</td>
<td>colon, liver</td>
<td>[Cheng et al., 2003]</td>
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</table>
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