The genetics of neutral lipid biosynthesis: an evolutionary perspective

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Turkish AR, Sturley SL. The genetics of neutral lipid biosynthesis: an evolutionary perspective. Am J Physiol Endocrinol Metab 297: E19–E27, 2009.—The storage of fatty acids and fatty alcohols in the form of neutral lipids such as triacylglycerol (TAG), cholesteryl ester (CE), and wax ester (WE) serves to provide reservoirs for membrane formation and maintenance, lipoprotein trafficking, lipid detoxification, evaporation barriers, and fuel in times of stress or nutrient deprivation. This ancient process likely originated in actinomycetes and has persisted in eukaryotes, albeit by different molecular mechanisms. A surfeit of neutral lipids is strongly, perhaps causally, related to several human diseases such as diabetes mellitus, obesity, atherosclerosis and nonalcoholic fatty liver disease. Therefore, understanding the metabolic pathways of neutral lipid synthesis and the roles of the enzymes involved may facilitate the development of new therapeutic interventions for these syndromes.

acyltransferase; triacylglycerol; wax ester; wax diester; cholesteryl ester

Neutral Lipids: What, Why, When, Where, And How?

Storage of fatty acids, fatty alcohols, and sterols in the form of neutral lipids serves to allocate resources for potential use in vital functions such as membrane formation, epidermal integrity, bile acid synthesis, lipoprotein trafficking, and sterologenesis. Neutral lipids, such as cholesteryl ester (CE), triacylglycerol (TAG), and wax ester (WE), provide organisms with inert forms of energy used in conditions of nutrient deprivation and environmental stress. They also provide an excellent “sink” to buffer the toxic effects of fatty acids and fatty alcohols. In times of plenty (such as a typical Western diet), energy in the form of fat is efficiently stored, but with consequences. Elevated cytoplasmic deposition of neutral lipids (primarily CE and TAG) is a significant risk factor for several disease pathologies, including diabetes, obesity, atherosclerosis, and nonalcoholic fatty liver disease (41, 53, 101). For example, the accumulation of CE in smooth muscle cells and macrophages in the vessel wall comprises the earliest recognizable stage in atherosclerotic plaque formation (90). Furthermore, serum levels of TAG and total cholesterol are independent risk factors for atherosclerosis. Similarly, the loading of TAG into the cytoplasm of adipocytes represents the basic unit of obesity and thus accounts for fat accumulation in all obese syndromes.

TAG, a fatty acyl ester derivative of glycerol, represents the major energy depot of all eukaryotic and some bacterial cells (16) and is formed primarily via an acyl-CoA diacylglycerol acyltransferase (DGAT) reaction, although in some organisms acyl-CoA-independent reactions also contribute to these pools. The energy of complete oxidation of the alkyl chains of TAG (38 KJ/g) is more than twice the same weight of carbohydrate or protein, and unlike polysaccharide, TAG carries no extra weight as water of solvation. Furthermore, TAG is deposited into cytoplasmic lipid droplets and thus has no effect on the osmolarity of the cytosol. This partitioning, which is common to all eukaryotic cells but particularly prevalent in adipocytes, is an efficient mechanism for safely and reversibly storing fatty acids and diacylglycerol (DAG) as well as providing important molecular precursors for membrane synthesis (4, 73). TAG has diverse roles throughout nature. In plants, it plays an important role in the germination of seeds. In mammals, it is involved in lipoprotein metabolism, fat storage, and milk production.

Similarly, the formation of CE is a critical homeostatic process that provides for storage of sterols, important molecules involved in membrane formation and stability that are toxic when in excess. In higher organisms, sterols are also precursors for steroid hormones and bile acids. The esterification of sterols with free fatty acids occurs via two pathways: 1) a subcellular acyl-CoA-dependent reaction involving acyl-CoA:cholesterol acyltransferases (ACATs) (15, 18, 76) or 2) an extracellular lecithin:cholesterol acyltransferase reaction that utilizes phospholipids as the acyl donor (39). In all eukaryotic cells, steryl esters (SEs) are stored in cytoplasmic lipid droplets. In mammalian liver or intestine, CE can also be packaged into lipoprotein particles in the endoplasmic reticulum for redistribution through the circulation. The esterification reaction thus represents a critical, evolutionary, conserved step in sterol homeostasis. However, the accretion of CE in macrophages or smooth muscle cells leads to the formation of foam cells along the arterial wall and has been implicated in the development of atherosclerotic lesions. This has sparked interest in the elucidation of the mediators and mechanisms of these reactions, with hopes of treating atherosclerosis and/or hypercholesterolemia.

WEs are neutral lipids composed of long-chain fatty alcohols esterified with fatty acids via the acyl-CoA:wax alcohol acyltransferase (AWAT) reaction. WE is abundantly found in the cuticle of plants, insect exoskeleton coating, spermaceti of...
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whales, and mammalian sebum and meibum. In addition to acting as another means to prevent free fatty acid-mediated toxicity, WEs also have diverse biological functions such as prevention of desiccation, infection, and ultraviolet light damage in plants, regulation of buoyancy of sperm whales, and an energy source in the seeds of the jojoba plant. WEs are under a unique market demand because they are an important component of cosmetics, polishes, coatings, and lubricants. The jojoba seed is of particular importance as a source of WE given the recent limited availability of oils from sperm whale hunting. WE also comprises a significant portion of mammalian sebum, the oil-rich secretions of the sebaceous gland within the skin. Although the function of sebum is largely unknown, it is clearly involved in epidermal homeostasis and the development of acne, the maintenance of fur hydration, and heat insulation in mammals (33, 98). The first acyltransferase for WE synthesis was identified from jojoba (54). To date, two mammalian AWATs have been described (27, 104, 117), both of which are highly expressed in the human sebaceous gland (104). Wax diesters are fatty acid derivatives of fatty alcohols. In addition to WEs and other neutral lipids, they comprise a critical portion of the tear film (68, 74). The meibomian glands, composed of a large row of holocrine glands that structurally and functionally are similar to the sebaceous gland, lie within the eyelids of mammals and secrete meibum, the lipid portion of the human tear film. This lipid component provides a smooth ocular surface for the cornea and prevents water evaporation from the ocular surface. Wax diesters have also been detected in various bacteria (51). They are also synthesized by the uropygial gland of high-Arctic-breeding sandpipers and temperate species during courtship until hatching (85, 87). It is suspected that the shift to diester preen waxes reduces birds’ smell and thereby reduces mammalian predation risk (86).

Neutral lipids are a heterogeneous array of different alcohols (sterols, diacylglycerols, long-chain alcohols) conjugated with fatty acids, wherein the extent of saturation and chain length, cyclic or linear, is manifold and likely imposes striking properties and functions. Thus, it is hardly surprising that multiple reactions and gene products govern their formation. In this review, we describe the impact and regulation of these reactions, focusing on their conservation across several billion years of evolution.

The Origins Of Neutral Lipids

The utility of TAG as both an energy source and a safe haven for toxic metabolites likely prompted the early evolution of acyltransferases that conjugate alcohols and fatty acids. The chronologically earliest reaction for acyl-neutralization of alcohols likely arose in bacteria (51), whereby long-chain alcohols or DAG are conjugated with fatty acids via the action of multifunctional WE synthases/acyl-CoA:diacylglycerol acyltransferases (WS/DGAT). TAG synthesis has also been found in prokaryotes such as Actinomycetes bacteria (2, 108) and the parasite Schistosoma Mansoni (7), where it serves primarily as an energy and carbon source. These genes do not exhibit any sequence conservation to eukaryotic acyltransferases involved in TAG or WE synthesis (46, 48–50, 100, 108). Recently, however, a novel plant WS/DGAT that possesses significant sequence identity to a similar bifunctional enzyme in A. calcoaceticus was identified (52).

In eukaryotes, at least three different acyltransferase gene families whose molecular and biochemical natures are different from prokaryotes have been identified. Biochemically, these three gene families encode two types of enzyme reactions, 1) acyl-CoA-dependent reactions integral to the intracellular membranes and 2) acyl-CoA-independent reactions that are peripherally associated with serum lipoproteins in which the acyl donor is a phospholipid. The acyl-CoA-dependent reactions are performed by two unrelated gene families (ACAT and DGAT2), that comprise as many as 10 different genes. Both ACAT and DGAT2 gene families are conserved across evolution, although with less internal redundancy. The acyl-CoA-independent reactions are also conserved from yeast through all metazoans. The prototype of an acyl-CoA-independent acyltransferase is lecithin cholesterol acyltransferase (LCAT), which acts as a phospholipase to cleave acyl groups from phospholipids (particularly phosphatidylcholine, also known as lecithin) and then donate them to sterols (103). In plants and fungi, members of the LCAT gene family encode intracellular proteins capable of synthesizing TAG and have been termed phospholipid:diacylglycerol acyltransferases (PDATs) (32).

Eukaryotic Ayltransferases

Two mammalian ACAT genes (ACAT1 and -2) have been identified and characterized (reviewed in Refs. 9, 25, and 79). ACAT1 is widely expressed with the highest levels in macrophages, steroidogenic tissues, sebaceous glands, and atherosclerotic lesions. ACAT2, on the other hand, is limited in its expression to the liver and small intestine (9, 79). The tissue distribution of the ACAT enzymes suggests that ACAT1 is involved primarily with deposition of CE into cytoplasmic lipid droplets, whereas ACAT2 is likely involved with lipoprotein assembly.

DAG is esterified to form TAG by the DGAT reaction (79). There are at least two independent mammalian enzymes known to catalyze this reaction, DGAT1 and DGAT2 (34). DGAT1 is structurally related to the ACATs, with the divergence in its amino acid sequence conferring its substrate specificity to DAG (16). Members of the DGAT2 family have no sequence homology to the ACAT family, including DGAT1. Three members of this family are autosomally encoded acyl-CoA:monoaeciglycerol acyltransferases (MGAT1, -2, and -3; reviewed in Ref. 25) that catalyze the synthesis of DAG by the esterification of monoaeciglycerol. It appears that the MGAT reaction provides an important alternative to the Kennedy pathway for the synthesis of DAG (and thus ultimately triglyceride) that could be particularly important for dietary fat absorption at the intestinal enterocyte.

Of interest, three other members of the DGAT2 gene family are clustered to a rather narrow X-linked locus of ~200 kbp; two encode AWAT1 and -2, respectively, which synthesize WEs by the esterification of long-chain fatty alcohols (104). The AWAT reaction appears to be particularly important in the sebocyte, because WE comprises a significant component of sebaceous gland secretions. The biochemical function for the final member of DGAT2 gene family (termed hDC3) remains to be determined. Sequence alignments reveal that the MGATs are evolutionarily closely related to each other, whereas
AWAT1, AWAT2, and hDC3 are clustered together (Fig. 1). The DGAT2 gene family members are likely derived from an ancestor with lysophosphatidic acid acyltransferase activity, a reaction that is required for the final steps in phosphatidic acid biosynthesis. This homology is maintained in the whole human DGAT2 gene family and includes the conservation of residues at the active sites of bacterial glycerol-3-phosphate acyltransferases. Both the ACAT and DGAT2 enzyme families are conserved across multiple organisms, including yeast and plants (79), as is the PDAT family.

In humans, DGAT1 is highly expressed in human small intestine, colon, testis, and skeletal muscle, with lower levels of expression in adipose and liver (16, 76). DGAT2 also possesses widespread expression in humans, with particularly high levels in liver and adipose tissue (17). DGAT2 has been shown to colocalize and interact with steroyl-CoA desaturase 1, an enzyme responsible for the formation of monounsaturated fatty acids (67). This suggests that DGAT2 plays an important role in the esterification of endogenously formed monounsaturated fatty acids. The expression and protein interaction patterns of the DGATs indicate that they may have different functions within different tissues. It is reasonable to speculate that DGAT1 likely plays a role in intestinal repackaging of free fatty acids using the monoacylglycerol pathway following exogenous fatty acid uptake or lipolysis of dietary TAG in the lumen of intestine, whereas DGAT2 may function primarily in de novo TAG synthesis and secretion from the liver into lipoproteins and deposition in adipose tissue.

Given the critical role that neutral lipids play in many cellular functions, it is not surprising that multiple biosynthetic pathways exist. What is the reason for the genetic redundancy within the DGAT2 gene family, then? Although all of its members possess DGAT activity in vitro, it has been postulated that the existence of multiple genes may be related to their specific tissue expression patterns and alternate substrate specificities. For example, mouse MGAT1 is expressed in most tissues but not in the intestine, suggesting that it may play an important role in tissues other than the small bowel (120). In contrast, MGAT2 and -3 are expressed primarily in the intestine and are likely the key contributors to TAG repackaging within the enterocyte (11, 13, 14, 26, 66, 118). Biochemically, MGAT3 prefers 2-monoacylglycerol, the predominant product of TAG lipolysis in the intestinal tract, but MGAT1 and -2 do not have such substrate specificity and use 1- and 3-monoacylglycerols equally well. Interestingly, MGAT3 is not found in lower mammalian species, and it is most abundantly expressed in the human ileum, which is distal to the regions of maximum lipid absorption. MGAT3 has also been shown to possess rather potent DGAT activity (12). Thus, the precise role of MGAT3 in lipid absorption remains to be determined.

The X-linked subgroup of the DGAT2 gene family is highly expressed in the human sebaceous gland and possesses significant but modest DGAT activity. However, two AWATs (AWAT1 and -2) mediate the synthesis of WEs (104), as does the murine ortholog of AWAT2 (27). The existence of two AWATs can be explained by their different expression profiles and substrate specificities. AWAT expression is limited to the sebaceous gland in a differentiation-specific manner (104). AWAT2 is restricted to undifferentiated peripheral sebocytes, whereas AWAT1 is expressed in more mature, centrally located cells. AWAT1 has a preference for shorter-chain fatty acids and saturated acyl groups, whereas AWAT2 prefers longer-chain fatty acids and unsaturated acyl-CoAs. This expression profile and substrate specificity pattern suggests that WE metabolism reflects and may play an important role in sebocyte differentiation. It is possible that, as the sebocyte matures, a cycle of WE lipolysis and reesterification occurs such that the WEs are remodeled prior to their secretion onto the skin. This may serve to homogenize the sebum in terms of fatty acid and alcohol saturation or chain length such that optimal hydrophobicity and effectiveness as a skin barrier is achieved.

**Model Systems For Studying Acyltransferases**

*Yeast genetic dissection of DAG and sterol acyltransferases.* The yeast *Saccharomyces cerevisiae* has provided an immensely valuable eukaryotic system to investigate neutral lipid metabolism and dissect its role in many cellular processes. In addition to identifying and characterizing members of the various human acyltransferase gene family members (59, 76, 104), we have identified and deleted the full complement of genes for TAG and SE synthesis in this model eukaryote (78, 93, 115) (Table 1). As a result, we have generated viable strains of yeast that lack neutral lipids and used these strains to define the activities of their human counterparts (76, 104, 116).

In yeast, ACAT-related enzymes 1 and 2 (*ARE1* and *ARE2*) are the orthologs of mammalian ACATs. Cells that lack *ARE1* and *ARE2* are unable to esterify sterols yet are viable due to adaptive downregulation of ergosterol synthesis. Whereas *ARE2* prefers ergosterol as its substrate, *ARE1* esterifies a wider range of sterols and may serve as both a detoxification and storage reaction for sterol biosynthetic intermediates (79).

In yeast, three structurally different enzymes arbitrate DAG esterification. In an acyl-CoA-independent manner, *LRO1*, an ortholog of mammalian LCAT, mediates TAG synthesis by utilizing phospholipids as acyl donors in a PDAT reaction (78).
DGAI, the sole yeast ortholog of human DGAT2, which utilizes acyl-CoA as the acyl donor, is responsible for the remaining TAG synthesis in cells that lack LRO1 (77). Whereas the LRO1 reaction predominates during the exponential growth phase of yeast, the DGAI-mediated DGAT reaction predominates during the relatively quiescent stationary phase of yeast. Deletion of LRO1 decreases TAG synthesis by ≈75% of the normal strain, whereas loss of DGAI significantly decreases TAG synthesis by ≈60%, depending on culture conditions. The yeast ortholog of DGAT1, ARE2, plays a minor role in TAG synthesis (76). Triple deletion of DGAI, LRO1, and ARE2 in the same strain eliminates detectable TAG synthesizing capabilities in yeast. Furthermore, deletion of ARE1 in such a strain obliterates the ability of such cells to synthesize SE and TAG. Subsequently, utilization of such strains devoid of neutral lipids has facilitated the study of mammalian enzymes involved in neutral lipid synthesis (79).

Fruit fly and nematode genetic models for obesity. The prevalence of obesity in Western populations and its recalcitrance to dietary intervention has prompted a quest for model systems in which to define its genetic origins (6). The majority of insight into this etiology has arisen from studies in inbred or recombinant inbred strains of mice that are resistant to diet-induced hypercholesterolemia and gallstone formation. As a result, they are protected from developing atherosclerosis in an apoE−/− background (8, 109).

The impact of DGAT1 and DGAT2 in mammalian TAG synthesis and energy homeostasis has also been highlighted in murine models that are deficient in these genes. Although mice lacking DGAT1 (Dgat1−/−) exhibit 50% less body fat and are otherwise healthy and fertile with normal serum TAG (95), they exhibit poor milk production due to deficient TAG production in mammary glands, dry fur, and atrophy of the sebaceous glands (95). Unexpectedly, the dry fur phenotype was associated with reduced levels of wax diesters, but not TAG, in the skin. This is consistent with in vitro assays suggesting that DGAT1 is capable of esterifying long-chain alcohols and dialcohols into WEs and diesters, respectively (76). Dgat1−/− mice provide evidence that DGAT1 plays an important role in retinol metabolism through its acyl CoA:retinol acyltransferase activity. DGAT1 is capable of esterifying retinol in vitro (75, 80, 119) and within the enterocyte in vivo (111). Furthermore, Dgat1−/− mice have greatly reduced liver, testes, and kidney acyl CoA:retinol acyltransferase activity (119) with increased levels of unesterified retinol in their livers when exposed to a high-retinol diet. Despite this, Dgat1−/− mice are resistant to high-fat diet-induced obesity and hepatic steatosis and have lower plasma glucose levels associated with increased insulin and leptin sensitivity (95). Furthermore, transplantation of white adipose tissue from Dgat1−/− mice into wild-type mice rendered wild-type mice resistant to obesity with improved insulin sensitivity (21). This suggests that adipocyte-derived factors are responsible for the improved body fat, glucose disposal, and energy expenditure in a DGAT1 deficiency state.

These approaches were complemented by studies in which DGAT1 was overexpressed either in cell culture or in specific tissues. Although mice that overexpress DGAT1 in white adipose tissue become obese due to increased adipocyte TAG deposition, they are insulin sensitive and have normal glucose disposal (22). Overexpression of human DGAT1 in rat hepatoma McA-RH7777 cells increased synthesis, cellular accumulation, and secretion of TAG (59). This is associated with decreased intracellular degradation and increased secretion of newly synthesized apolipoprotein B (apoB). Consistent with these findings, mice overexpressing hepatic DGAT1 exhibit increased VLDL secretion from the liver (114). These studies suggest that the liver is capable of adequately compensating for enhanced hepatic DGAT1 expression. It has been shown, though, that short-term hepatic overexpression of DGAT1 of mice have been created via transgenic approaches. Phenotypic characterization of these strains has greatly enhanced our knowledge about the physiological functions of these enzymes. Deletion of ACAT1 in mice has no effect on serum cholesterol, but curiously, it promotes atherosclerosis (1, 35). ACAT1-null mice (Acat1−/−) also suffer from dry eyes due to atrophic meibomian glands and, when crossed with low-density lipoprotein receptor-deficient (LDLR−/−) or apolipoprotein E (apoE)-knockout (ApoE−/−) mice, are prone to toxic accumulation of free cholesterol in the brain and the development of extensive cutaneous xanthomas due to cholesterol deposition in the skin (112). In contrast, mice lacking ACAT2 (Acat2−/−) have impaired dietary cholesterol absorption and are resistant to diet-induced hypercholesterolemia and gallstone formation. As a result, they are protected from developing atherosclerosis in an apoE−/− background (8, 109).

Reverse mammalian genetic models of acyltransferases. After the molecular identification of genes for the enzymes involved in neutral lipid synthesis pathways, numerous strains

### Table 1. Neutral lipid gene families are conserved from humans to yeast

<table>
<thead>
<tr>
<th>Gene Family</th>
<th>Gene Code</th>
<th>Species</th>
</tr>
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<tbody>
<tr>
<td>ACAT</td>
<td>Acat1</td>
<td>Human</td>
</tr>
<tr>
<td>DGAT1</td>
<td>Dga1</td>
<td>Yeast</td>
</tr>
<tr>
<td>DGAT2</td>
<td>Lro1</td>
<td>Yeast</td>
</tr>
<tr>
<td>LCAT</td>
<td>Lcat1</td>
<td>Yeast</td>
</tr>
</tbody>
</table>

ACAT, acyl-CoA:cholesterol acyltransferase; DGAT, diacylglycerol acyltransferase; LCAT, lecithin cholesterol acyltransferase; DGAT1, the sole yeast ortholog of human DGAT2; LRO1, the yeast ortholog of the human LCAT; ARE, ACAT-related enzyme.
increases the TAG content in the liver but not VLDL production (71).

In contrast to the healthy physiological changes associated with DGAT1 deficiency, Dgat2−/− mice die in the neonatal period as a result of severe skin permeability defects and metabolic decompensation associated with marked depletion of TAG and free fatty acids in their tissues and plasma (99). DGAT1 could not compensate for the loss of DGAT2, supporting the hypothesis of different functions for the two enzymes. Thus, it appears that DGAT2 is the enzyme responsible for the majority of TAG synthesis in mice. Although DGAT2 deficiency appears to be maladaptive, when hepatic DGAT2 expression is knocked down by administration of DGAT2-specific antisense oligonucleotides in wild-type and ob/ob obese mice on high-fat diets, there is a marked reduction in hepatic TAG content and steatosis (121). Of note, when DGAT2 antisense oligonucleotides were administered to wild-type and Dgat1−/− mice, VLDL secretion was reduced in a dose-dependent manner, supporting the hypothesis that DGAT2 is responsible for TAG that is destined for hepatic secretion (65). Thus, DGAT2 may be a legitimate target for dyslipidemic disorders such as nonalcoholic fatty liver disease.

### Regulation of Acyltransferase Reactions

It has been shown that the ACATs are regulated by translational and posttranslational mechanisms. ACAT1 mRNA is increased in the liver and aorta following a cholesterol rich diet (82, 105) or after HepG2 cells are exposed to free fatty acids (93). For the most part, however, ACAT1 is allosterically activated by cholesterol and oxysterols (24) but not fatty acids (19). Given the degree of sequence conservation, it seems likely that ACAT2 is also allosterically regulated by cholesterol, although transcriptional regulation of this isoform has been observed (20, 61, 96, 107).

The DGAT reaction provides a switch point for the allotment of the cell’s DAG resources because it lies at a branch point where DAG is used for the synthesis of phospholipid or TAG. Consistent with this hypothesis, DGAT1 mRNA levels increase sevenfold when 3T3-L1 cells are induced with insulin and dexamethasone to differentiate into adipocytes. This yields an ∼90-fold increase in DGAT1 protein and DGAT activity (122). However, when DGAT1 is overexpressed in undifferentiated 3T3-L1 cells, a 20- to 40-fold increase in mRNA is associated with a modest two- to threefold increase in DGAT activity, suggesting that DGAT1 is rate limiting in TAG synthesis. DGAT2 expression also increases 30-fold during the differentiation of 3T3-L1 cells; its expression is increased further when the cells are treated with glucose and insulin (70). It appears that glucose preferentially enhances DGAT1 mRNA expression, whereas insulin increases the level of DGAT2 mRNA. However, when fasting mice were refed with a high-carbohydrate meal, DGAT2, but not DGAT1, mRNA was increased in liver, adipose, and small intestine.

There is modest evidence to date that suggests that DGAT1 and DGAT2 are regulated by specific transcription factors. Thiazolinediones, which activate peroxisome proliferator-activated receptor-γ, increase DGAT1 mRNA in adipose cells and tissues (84). CCAAT/enhancer-binding proteins (C/EBPβ and C/EBPα) have been shown to increase expression of DGAT2 during adipogenesis (83). Recently, there has been increased interest in the unfolded protein response as a consequence of cellular stress and as an inducer of de novo lipogenesis. X-box-binding protein-1 regulates the unfolded protein response and has been shown to increase DGAT2 expression in the liver, whereas lack of hepatic X-box-binding protein-1 leads to a decrease in liver lipid content (55). DGAT1 and -2 may also be regulated by the MEK/ERK signaling pathway (102).

### Pathophysiologic Consequences of Aciytransferase Reactions

Unesterified alcohols (cholesterol and DAG) and free fatty acids are ultimately lipotoxic to all eukaryotic cells, and it is their deposition as neutral lipids [primarily as SE and triglyceride (TG)] into cytoplasmic lipid droplets that presents a safe haven for these bioactive molecules. Counterintuitively, neutral lipid deposition is highly associated with several diseases, such as obesity, diabetes, atherosclerosis, and nonalcoholic fatty liver disease. Neutral lipid biosynthesis thus represents an important facet of lipid homeostasis when reaction substrates are in excess (i.e., a typical developed-world diet). Although the pathophysiological ramifications of elevated neutral lipids are clear, the importance of these molecules and the cytoplasmic lipid droplets in which they reside in an optimal, lipid-balanced cell is unknown.

Studies in which neutral lipid-synthesizing enzymes are overexpressed highlight the consequences of neutral lipid overload. When human ACAT1 is overexpressed in the liver of Ldlr−/− mice, hepatic CE increases with a consequent increase in plasma VLDL, cholesterol, and TAG (97). In cultured McA-RH7777 cells, overexpression of human ACAT1 or ACAT2 led to an increase in synthesis, cellular accumulation, and secretion of CE as well a decrease in degradation of VLDL apoB (59). These studies indicate that CEs formed by the ACATs play an important role in regulating the assembly and secretion of apoB-containing lipoproteins. Recently, it has also been shown that the diabetes-induced dyslipidemia seen in streptozotocin-induced diabetic rats is partly due to an increase in intestinal ACAT2 activity (45).

Several studies have documented the role of hepatic DGAT in the development of hepatic steatosis. Mice overexpressing DGAT1 in the liver exhibit increased hepatic VLDL secretion and, interestingly, increased gonadal but not subcutaneous fat (114). In the same study, mice that overexpress DGAT2 in the liver accumulate significant TAG. This agrees with the hypothesis that DGAT1 plays a larger role in VLDL assembly, whereas DGAT2 fuels cytosolic TAG accumulation and the development of steatosis. Short-term hepatic overexpression of DGAT1 and DGAT2 increases liver TAG content, but with no effect on VLDL production (71). In both overexpression models, cytosolic but not microsomal lipid content significantly increased. This suggests that, in the presence of adequate hepatic lipid stores, factors other than TAG synthesis might be rate limiting in VLDL production. In another study, overexpression of DGAT2 in mouse liver led to steatosis without the development of insulin resistance, indicating that hepatic steatosis can occur independently of insulin resistance (72). This suggests that factors other than TAG content may be required for the development of insulin resistance. Thus, the precise role...
of the DGATs in hepatic TG metabolism and the development of nonalcoholic fatty liver disease have yet to be determined.

Despite the myriad of disorders that are associated with neutral lipid accumulation, recent evidence suggests that neutral lipids may have important cytoprotective functions (103). Free fatty acids, sterols, and potentially DAG are toxic yet essential components of all eukaryotic cells. Thus, for each of these molecules there are complex, often overlapping, mechanisms that function to optimize their levels. This homeostasis comprises several layers of regulation, including the control of synthesis (transcriptional and/or posttranslational), export, and, finally, detoxification by the acyltransferases described here. When these checks and balances are awry, lipotoxicity ensues. For example, when the fission yeast Schizosaccharomyces pombe (123) lacks the enzymes responsible for TAG synthesis, apoptosis results from an accumulation of long-chain saturated fatty acids such as palmitate and stearate. Denudation of TAG synthesis machinery and the TAG droplets render these cells sensitive to all fatty acids, including the monounsaturated free fatty acids such as oleate. In addition, strains of Saccharomyces cerevisiae lacking all neutral lipids display hypersensitivity to unsaturated fatty acids (40). In this model, cell death involves activation of stress pathways such as the unfolded protein response, culminating in apoptosis. This is markedly similar to the induction of lipopoptosis in many mammalian cell types, including pancreatic β-cells and rat hepatoma cells (31, 81). Through these studies, it has been demonstrated that neutral lipid biosynthesis is a protective step against lipotoxicity (64, 91).

Consistent with this hypothesis, excess fatty acid accumulation in Dgat1−/− fibroblasts leads to lipotoxicity and cell death (62). In addition, transgenic mice that overexpress DGAT1 in white adipose tissue become obese due to adipocyte TAG deposition but surprisingly are insulin sensitive with normal glucose disposal and an unchanged hepatic TAG content despite elevated plasma free fatty acids (22). Adipose-specific DGAT1 overexpression, then, may protect the liver by shuttling lipotoxic free fatty acids toward TG synthesis in adipose rather than insulin-sensitive tissues such as the liver. The protective role of neutral lipids has also been highlighted in lipodystrophic individuals, who lack adipose depots for TAG and are insulin resistant, with type II diabetes, dyslipidemia, and marked hepatic steatosis (94). Consistent with this, when DGAT1 is overexpressed in adipocytes of obese-resistant FVB mice, marked hepatic steatosis develops in association with obesity resistance, elevated plasma free fatty acids, and insulin resistance (23). It appears, then, that insulin resistance and hepatic steatosis is more closely related to increased exposure to free fatty acids and TG accumulation in insulin-sensitive tissues such as the liver, rather than adipose.

Recently, it has also been shown that, although antisense oligo-mediated hepatic DGAT2 knockdown reduces steatosis, improves insulin sensitivity, and reduces weight in obese nonalcoholic steatohepatitis-susceptible mice, it is associated with markedly increased hepatic free fatty acids, elevated serum transaminases, lobular necroinflammation, and exacerbation of fibrosis (113). Markers of lipid peroxidation and oxidative stress, the second “hit” in nonalcoholic fatty liver disease, are markedly increased in this model despite a decrease in hepatic expression of tumor necrosis factor-α. It is possible, then, that hepatolipotoxicity and progression to fibrosis develops when the cellular capacity for free fatty acid deposition is overwhelmed or when TG pools are hydrolyzed, increasing exposure to toxic free fatty acids.

Lipotoxicity is also associated with cardiomyopathy, type 1 and 2 diabetes, congenital and acquired lipodystrophies, and obesity. Excessive free fatty acid levels characterize these syndromes, and the capacity to store these molecules as TAG in adipose tissue likely represents an important survival step. Conversely, the induction of lipoapoptosis in nonadipose tissues such as the pancreas or the heart in response to free fatty acid accumulation may be a major factor in diseases associated with overnutrition and old age (106). The cytotoxicity of saturated fatty acids such as palmitate parallels the elevated ceramide levels and/or reactive oxygen species (44, 63). On the other hand, the monounsaturated fatty acid oleic acid is the most abundant fatty acid in adipose tissue (47) and has been reported to promote apoptosis through activation of serine/threonine protein phosphatases as PP2Ca/b (92). Therefore, saturated and unsaturated fatty acids are both proapoptotic in several cell types (69), but each may achieve its effect through different mechanisms. In addition, increasing evidence indicates that endoplasmic reticulum stress and induction of the unfolded protein response might also be involved in the lipotoxic pathway for both saturated and unsaturated fatty acids (5, 81).

Conclusions

The persistence of neutral lipids, both through evolution and in different cell types, and the manner in which several different acyltransferase reactions have arisen undoubtedly reflects their physiological importance. It is our perspective that the selective advantage conferred by these genes represents primarily a protection from the lipotoxicity of the substrates that are themselves frequently essential. The products of the reactions have subsequently acquired functions that are independent of lipid storage, often as permeability barriers in the skin and the cornea and perhaps other tissues in mammals and the cuticle of plants. Interestingly, a common defect in animals lacking any one of the acyltransferases is loss of integrity of the epidermis. This is despite the apparent redundancy of these gene families, suggesting that the composition of these oils is just as relevant as overall levels.

The multiplicity of genes involved (for example, 3 ACAT and 7 DGAT2 genes in humans) reflects gene duplication events that have then conferred the ability to esterify alternate alcohols, possibly with tissue-specific patterns and in response to different stimuli (Table 2). Although the DGAT2 gene family members are a classic example of physiological redundancy, at the same time the metabolically relevant function of each is still not understood. This may reflect the fact that they are all involved in the lipotoxic pathway and have other roles.

Table 2. Primary activities of human neutral lipid acyltransferases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Preferred Substrate</th>
<th>Product</th>
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</thead>
<tbody>
<tr>
<td>ACAT1 and -2</td>
<td>Free cholesterol</td>
<td>Cholesterol ester</td>
</tr>
<tr>
<td>DGAT 1 and -2</td>
<td>Diacylglycerol</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>AWAT1</td>
<td>Short-chain alcohols</td>
<td>Wax ester</td>
</tr>
<tr>
<td>AWAT2</td>
<td>Long-chain alcohols</td>
<td>Wax ester</td>
</tr>
<tr>
<td>MGAT1 and -2</td>
<td>1- and 2-Monoacylglycerol</td>
<td>Diacylglycerol</td>
</tr>
<tr>
<td>MGAT3</td>
<td>2-Monoacylglycerol</td>
<td>Diacylglycerol</td>
</tr>
</tbody>
</table>

AWAT, acyl-CoA:wax alcohol acyltransferase; MGAT, acyl-CoA:monoacylglycerol acyltransferase.
dancy, the precise mechanisms by which they are regulated and expressed within specific tissues remain to be determined. If the reactions of the DGAT2 gene family products proceed in a similar manner to other acyltransferases, one may postulate that their mechanisms of regulation are similar. Alternatively, their varying expression and substrate specificity patterns may allude to different means of regulation. These factors remain to be determined. Just as obscure is the nature of the active sites that determine substrate specificity. The elucidation of either of these aspects of neutral lipid biosynthesis represents an untapped avenue of pharmacology that could make a significant impact in treatment of several human disease syndromes.

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REFERENCES


