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Glycerol-3-phosphate acyltransferases: Rate limiting enzymes of triacylglycerol biosynthesis

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Summary

Four homologous isoforms of glycerol-3-phosphate acyltransferase (GPAT), each the product of a separate gene, catalyze the synthesis of lysophosphatidic acid from glycerol-3-phosphate and long-chain acyl-CoA. This step initiates the synthesis of all the glycerolipids and evidence from gain-of-function and loss-of-function studies in mice and in cell culture strongly suggests that each isoform contributes to the synthesis of triacylglycerol. Much work remains to fully delineate the regulation of each GPAT isoform and its individual role in triacylglycerol synthesis.

Keywords

Glycerolipid; phospholipid; membrane; lipid droplet; lysophosphatidic acid; diacylglycerol

Glycerol-3-phosphate acyltransferases are members of the pfam 01553 family of acyltransferases

After Eugene Kennedy and his colleagues showed that the esterification of glycerol-3-phosphate with a long-chain acyl-CoA was the initial step in the synthesis of phospholipids [1] and triacylglycerol (TAG) [2], Pullman's group reported that this *sn*-glycerol-3-phosphate acyltransferase activity (GPAT; EC 2.3.1.15) was comprised of what appeared to be two isoforms, one located in the mitochondrial outer membrane and the other in the endoplasmic reticulum [3]. The endoplasmic reticulum (microsomal) activity was inhibited by sulfhydryl reagents such as *N*-ethylmaleimide (NEM) and exhibited no preference for particular acyl-CoA species, whereas the mitochondrial activity was resistant to NEM inactivation and preferred to use saturated acyl-CoAs like 16:0-CoA and 18:0-CoA [4]. With the identification of four genes encoding separate GPAT isoenzymes [5–11], we now know that GPAT mediated regulation of glycerolipid synthesis is more complex than anyone had previously thought; investigators are currently struggling with the question as to why four separate isoforms are required for glycerolipid biosynthesis.

Gpat1, the first mammalian GPAT isoform cloned [5,6], resides in the outer mitochondrial membrane, is resistant NEM inactivation and prefers to use saturated acyl-CoAs [4]. A second mitochondrial GPAT, GPAT2, also resides in the outer mitochondrial membrane, but its activity is inhibited by NEM and it has no long-chain acyl-CoA preference [12]. The NEM-

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sensitive endoplasmic reticulum isoforms, GPAT3 and GPAT4, were identified very recently [9–11].

All four GPAT isoforms are members of the pfam 01553 family of glycerolipid acyltransferases and contain four conserved motifs first identified by a bioinformatics approach [13] (Figure 1). Mutagenesis of invariant residues in these motifs verified that these highly conserved regions are essential for the activity of *E. coli* GPAT (PlsB) [14,15], mouse GPAT1 [16], and human dihydroxyacetone phosphate acyltransferase (DHAPAT) [17]. Residues important for catalysis are the invariant histidine and aspartate in Motif I, the phenylalanine, glutamate, glycine, and arginine in Motif III, and the proline in Motif IV [15,16,18]. Amino acids important for binding *sn*-glycerol-3-phosphate are the phenylalanine and arginine in Motif II, and glutamate and serine in Motif III [15,16,18]. A diverse array of acyltransferases have been assigned to pfam 01553 and activities have been verified biochemically for GPAT1 - 4, *sn*-1-acylglycerol-3-phosphate O- acyltransferase (AGPAT) -1 and -2, DHAPAT, lysophosphatidylcholine acyltransferase (LPCAT) -1, -2 and -3, lysophosphatidylglycerol acyltransferase (LPGAT), and acyl-CoA:lysocardiolipin acyltransferase (ALCAT) [4,17,19–25]. In spite of its homology to the glycerolipid acyltransferases, tafazzin, a protein required for the synthesis of normal cardiolipin, exhibits a transacylase activity [26–28]. A fifth motif present in the AGPAT isoforms, GPAT-3 and -4, and tafazzin has been inferred because amino acid mutations in this region of human AGPAT2 and in tafazzin cause congenital generalized lipodystrophy or Barth syndrome, respectively [29,30]. Amino acids important for the specialized activities of most of the other pfam 01553 family members have yet to be identified.

GPAT1 is an important regulator of TAG synthesis

Although GPAT1 resides in the outer mitochondrial membrane and diacylglycerol acyltransferase, the terminal enzyme of TAG synthesis, resides on the ER, many studies support an important role for GPAT1 in regulating TAG synthesis. GPAT1 activity is highest in rat liver and adipose, tissues with a high capacity for TAG synthesis [12]. In these tissues, GPAT1 activity is modulated in a manner consistent with the regulation of TAG synthesis. For example, in rat liver and adipose tissue, a 48 h fast decreases GPAT1 protein expression and activity more than 30%. When fasted rats are refed sucrose, GPAT1 protein expression and activity increases greater than 2-fold in liver [31,32], and in perfused rat liver, insulin increases GPAT1 activity 34% [33]. Similarly, streptozotocin-induced diabetes decreases GPAT1 activity in rat epididymal fat 60%, and insulin administration restores GPAT activity [34].

Changes in GPAT1 activity are mediated transcriptionally. When fasted mice are refed a high-carbohydrate diet, hepatic *Gpat1* mRNA levels increase 20-fold due to enhanced transcription rates [31]. The refeeding-induced increase in *Gpat1* message occurs in streptozotocin-diabetic mice only if insulin is administered. This regulation of *Gpat1* by insulin is mediated by sterol regulatory element binding protein-1 (SREBP-1), a transcription factor responsible for transactivating numerous genes required for enhanced fatty acid and TAG synthesis [35]. Insulin induces transcription of SREBP-1c, and in a typical counter-regulatory fashion, glucagon via cAMP opposes the action of insulin [36]. SREBP-1c appears to be the primary transcriptional regulator of GPAT1. The mouse *Gpat1* promoter region, first characterized by Jerkins et al. [37], contains three SREBP-1 consensus sites which are responsible for SREBP-1 mediated transactivation [38]. When SREBP-1c is ectopically expressed in 3T3-L1 preadipocytes, *Gpat1* mRNA expression increases 6.7-fold [38]. Conversely, in SREBP-1 knockout mice, the normal 8-fold induction of hepatic *Gpat1* mRNA expression by refeeding is abolished [39]. Although LXR agonists have been reported to upregulate GPAT1, LXR does not directly transactivate *Gpat1*, but rather upregulates SREBP-1c by a transcriptional mechanism [40]. In addition, although *Gpat1* mRNA increases with carbohydrate feeding, there is no evidence that this upregulation is mediated through the carbohydrate response

element; instead, it appears to proceed entirely through insulin-mediated transactivation of SREBP-1c since *Gpat1* mRNA expression is similar in wildtype and *Chrebp*^{-/-} mice [41].

The studies summarized above indicate that GPAT1 in liver is regulated in a manner consistent with a role in initiating TAG synthesis when an animal is presented with excess carbohydrate or fat calories. Less information is available about the regulation of GPAT1 in other tissues. *Gpat1* mRNA increases 10-fold when 3T3-L1 cells differentiate into adipocytes [5], coinciding with an increase in TAG synthesis, and mitochondrial GPAT activity (probably GPAT1) decreases 50% in rat epididymal fat and liver, but not in gastrocnemius muscle, after treadmill exercise, probably regulated by AMP-activated kinase (AMPK) [42]. However, NEM-resistant GPAT activity increases in brown adipose tissue (BAT) when animals are exposed to cold [43], suggesting that TAG synthesis may continue simultaneously with TAG lipolysis. Such a futile cycle would contribute to the energy wasting and heat production in BAT. None of these effects, however, has been examined systematically and the controlling factors have not been elucidated.

The role of GPAT1 in TAG synthesis

Gain-of-function and loss-of-function studies highlight the importance of GPAT1 in *de novo* TAG synthesis. Plasmid- and adenovirus-mediated overexpression of GPAT1 in CHO and HEK293 cells and in primary rat hepatocytes increases TAG content and oleate incorporation into TAG 3- to 4-fold [44,45]. Further, compared to rats infected with a control virus, when rats are infected with a GPAT1 expressing adenovirus, NEM-resistant GPAT activity, hepatic TAG content, and plasma TAG concentration each increase about 2.7-fold [46]. In contrast, compared to wildtype controls, female mice that lack GPAT1 (*Gpat1*^{-/-}) weigh less, have smaller gonadal fat pads, have 40% lower hepatic TAG content, 15% lower plasma TAG, and 30% reduced secretion of VLDL-TAG [47]. Lipogenic diets also result in less TAG accumulation in liver and heart from *Gpat1*^{-/-} mice [48,49] (T.M. Lewin, unpublished), although the total weight gain of *Gpat1*^{-/-} and wildtype mice is similar. Consistent with a role for GPAT1 in mediating TAG synthesis, a 90% adenovirus-mediated shRNA knockdown of liver *Gpat1* in *ob/ob* mice reduces liver TAG content 30–42% within 5 days [50]. Surprisingly in this model, no change was observed in plasma TAG concentration.

GPAT1 regulates acyl-CoA metabolism at the mitochondrial membrane

In addition to controlling the flux of acyl-CoAs that enter the pathway of TAG synthesis, GPAT1 also regulates acyl-CoA use for β -oxidation. Once fatty acids are activated to acyl-CoAs, they can be esterified by GPAT and enter the pathway of glycerolipid biosynthesis or they can be converted to acyl-carnitines by carnitine palmitoyltransferase-1 (CPT1) and then enter the mitochondrion for β -oxidation. GPAT1 and CPT1 are intrinsic proteins of the outer mitochondrial membrane, and appear to compete for the same long-chain acyl-CoA pool in order to channel acyl-CoAs towards the synthetic or degradative pathways. This reciprocal channeling of acyl-CoAs is observed in *Gpat1*^{-/-} livers which contain elevated acyl-CoAs and acyl-carnitines and show increased ketogenesis [48]. Conversely, adenovirus mediated overexpression of GPAT1 in hepatocytes diminishes [¹⁴C]oleate oxidation 40% while increasing [¹⁴C]oleate incorporation into TAG more than 2-fold [12].

AMPK acutely partitions fatty acids to degradative vs. biosynthetic fates. AMPK is active when energy stores are deficient or when energy demand is high [51]. High AMPK activity favors catabolic processes that supply ATP while inhibiting biosynthetic reactions that use ATP. Thus, active AMPK phosphorylates and inhibits acetyl CoA carboxylase, decreasing cellular malonyl-CoA, and relieving the malonyl-CoA inhibition of CPT1 [51]. Because CPT1 is the rate-limiting step for β -oxidation, increased CPT1 activity results in enhanced fatty acid catabolism. Concomitantly, activated AMPK decreases GPAT1 activity and diminishes the

use of acyl-CoAs for TAG synthesis. Specifically, both 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside, a nucleoside activator of AMPK, and recombinant AMPK inhibit GPAT activity 30–40% in hepatocytes and muscle [52]. In hepatocytes, AICAR treatment diminishes [14 C]oleate incorporation into TAG 40% [52]. Thus, the actions of AMPK contribute to the acute reciprocal and coordinated regulation of acyl-CoA channeling at the outer mitochondrial membrane. Although GPAT1 contains several putative AMPK phosphorylation sites, it is not known whether AMPK directly phosphorylates GPAT1.

Casein kinase (CK2) phosphorylates GPAT1 and enhances GPAT1 activity 50–200% in liver [53] and in T-lymphocytes [54]. Because CK2 also activates liver CPT1 20–25% and diminishes CPT1 inhibition by malonyl-CoA [55], it appears that unlike AMPK, CK2 does not reciprocally regulate fatty acid degradation and esterification. Further studies will be required to elucidate the physiological significance of CK2 phosphorylation of GPAT1 in the face of simultaneous activation of CPT1.

GPAT1 regulates the fatty acid composition of phospholipids

In addition to initiating TAG synthesis, GPAT1 also affects the synthesis of phospholipids. Consistent with the preference of GPAT1 to esterify palmitate at the *sn*-1 position of glycerol-3-phosphate, liver TAG and total phospholipids from *Gpat1*^{-/-} mice contain 40% and 35% less 16:0, respectively, than lipids from control mice [47]. In these mice the major phospholipids in liver, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) contain 20% less 16:0 at the *sn*-1 position. In place of 16:0, PE contained 30% more 18:0 and 64% more 18:1. Changes in PC were similar, with 44% more 18:0 found at the *sn*-1 position. These changes in the amount of 16:0 at the *sn*-1 position resulted in an altered composition of fatty acids found at the *sn*-2 position. For PE from *Gpat1*^{-/-} liver, the *sn*-2 position contained 36% more 20:4, 75% more 20:5, and 28% less 22:6 than wildtype mice. Similar results were found at the *sn*-2 position of PC which contained 40% more 20:4, 105% more 20:5, and 18% less 22:6. These data suggest that alterations in GPAT1 activity can alter both phospholipid fatty acid composition and cellular TAG content. Because PC is the primary component of the lipid monolayer surrounding lipid droplets, changes in PC fatty acid composition could alter lipid droplet structure.

GPAT2 has a minor role in initiating TAG synthesis

GPAT2 was first recognized in liver mitochondria from *Gpat1*^{-/-} mice because a polyclonal antibody raised against full-length GPAT1 identified a protein of similar molecular mass in liver from GPAT1 null mice [12]. Characterization of GPAT2 activity in liver mitochondria from *Gpat1*^{-/-} mice showed that, unlike GPAT1, GPAT2 is sensitive to inactivation by NEM, and has no preference for 16:0-CoA compared with oleoyl-CoA [12]. Subsequent cloning of *Gpat2*, showed that the mRNA transcript was highly abundant in testis and relatively low in other tissues [7]. When GPAT2 was overexpressed in Cos-7 cells, 84% more oleate was incorporated into TAG than in control transfected cells [7]. Although studies in cultured cells indicate that GPAT2 can initiate TAG synthesis, GPAT2 does not substitute functionally for GPAT1. For example, despite being active in *Gpat1*^{-/-} mice [12], the presence of GPAT2 does not prevent the diminished amount of TAG in liver [47]. Consistent with this finding is that *Gpat2* mRNA abundance is not altered by fasting or refeeding [7] as is observed for *Gpat1* and other lipogenic enzymes like fatty acid synthase and acetyl-CoA carboxylase [56]. The function of GPAT2 in testes mitochondria remains unexplored.

Microsomal GPAT isoforms

Until recently, it was believed that only a single NEM-sensitive (microsomal) GPAT existed and most studies earlier than 2007 could report only on enzyme activity. In both developing

neonatal rat liver [57] and differentiating 3T3-L1 adipocytes [58], the microsomal activity increases 70-fold, but the microsomal GPAT activity seemed to be less influenced by hormonal and nutritional status than GPAT1. For example, activation of AMPK in rat hepatocytes decreases mitochondrial GPAT activity without affecting microsomal GPAT [59], and work by Saggerson's lab in the 1970's showed that the NEM-insensitive GPAT (presumably GPAT1) activity in rat liver is increased by insulin [60] and decreased by starvation [61], whereas the microsomal activity did not change. In adipose tissue however, other studies showed inactivation of microsomal GPAT activity by a tyrosine kinase and activation by insulin or sodium orthovanadate [62–65].

Now that two ER-located GPAT isoforms, GPAT3 and GPAT4, have been cloned, the specific regulation of these isoforms must be reinvestigated. It will be important to reevaluate specific regulation that may increase or decrease only a single isoform when TAG formation is stimulated or inhibited. Because the microsomal isoforms are differently expressed in rodent liver and in specific adipose depots, it is possible that changes in one of these isoforms or opposite changes in both of them resulted in little change in total GPAT activity, thereby misleading previous investigators.

Glycerol-3-phosphate acyltransferase-3

The gene encoding *Gpat3* was mislabeled in GenBank as AGPAT8 (LPAAT-theta), based on sequence homology to AGPAT1 and 2. Cao and colleagues identified GPAT3, based not only on sequence similarities to acyltransferases, but also by cross-referencing genes that increased during adipocyte differentiation [9], because microsomal GPAT activity had been reported to increase 70-fold during 3T3-L1 differentiation [58]. Mouse and human *Gpat3* encode 438- and 434- amino acid proteins, respectively, both with an approximate molecular mass of 50 kDa and exclusively expressed in the endoplasmic reticulum [9]. In differentiated 3T3-L1 adipocytes, siRNA-mediated knockdown showed that GPAT3 (60% of mRNA) accounts for nearly 55% of total GPAT activity [9]. Further, *Gpat3* mRNA expression increases 60-fold in adipocytes after differentiation, and the *Gpat3* transcript increases 4.5-fold in white adipose tissue from mice treated with rosiglitazone [9], consistent with the role of PPAR γ in the regulation of lipogenic genes. *Gpat3* is highly expressed in epididymal adipose tissue as well as small intestine in mice, and in kidney, testis, heart, skeletal muscle, and thyroid in humans [9,66].

Glycerol-3-phosphate acyltransferase-4

Gpat3 expression is relatively low in livers from both mice and humans [9], whereas NEM-sensitive, microsomal GPAT activity accounts for at least 50% of the total GPAT activity in liver [4]. These data suggested that additional microsomal GPAT isoforms existed, and because GPAT3 shared 86.6% identical amino acid residues with a closely related protein, a fourth member of the GPAT family, GPAT4 was discovered [10]. Like GPAT3, GPAT4 was misidentified as an AGPAT (AGPAT6 or LPAAT-zeta in GenBank) based on sequence homology. GPAT4, which is located exclusively in the endoplasmic reticulum, has 456 amino acids and a predicted molecular mass of 52.2 kDa, but is expressed as a 48 kDa protein, likely due to cleavage of a 38 amino acid signal peptide [67–69]. Like GPAT3 [9], GPAT4 can use a range of acyl-CoA substrates from 12 to 20 carbons, however the highest GPAT activity occurs with acyl-CoA species of 16- and 18-carbons, regardless of the level of saturation [10,11]. Consistent with GPAT's role in initiating TAG synthesis, *Gpat4* mRNA is highly expressed in mouse lipogenic tissues, including liver and both BAT and visceral white adipose tissues, including omental, epididymal, and retroperitoneal depots [68]. *Gpat4* mRNA is also highly expressed in mouse testis [68].

In the liver, *Gpat4*^{-/-} (formerly termed *Agpat6*^{-/-}) mice have 49% lower total GPAT activity than wildtype mice and 65% less NEM-sensitive GPAT activity [10]. In BAT, loss of *Gpat4* accounted for 50% of total GPAT activity and 65% of NEM-sensitive GPAT activity. However, no changes in NEM-sensitive GPAT activity were observed in parametrial white adipose tissue [10]. These results suggest that more than one NEM-sensitive GPAT functions in the liver and BAT and that different fat depots contain different complements of GPAT isoforms. Although *Gpat3* is moderately expressed in the liver and BAT [9], its specific activity is unknown; therefore the relative contributions of GPAT3 and GPAT4 to NEM-sensitive GPAT activity in normal liver and BAT remain unclear. Importantly, no decrease in AGPAT activity was detected in tissues from the *Gpat4*^{-/-} mice, solidifying the discovery of GPAT4 as the enzyme previously identified as AGPAT6 [10].

Suggesting an important role for GPAT4 in TAG synthesis, *Gpat4*^{-/-} mice have a 45% lower content of TAG in liver and, when fed a chow diet, have a significantly decreased TAG content of both BAT and gonadal white adipose depots [68]. The reduction of adipose tissue contributes to the ~25% decrease in body weight but does not explain it fully. Further, *Gpat4*^{-/-} mice are protected from both high fat/high carbohydrate diet-induced obesity and from genetic obesity when they are crossed with obese, leptin deficient *ob/ob* mice [68]. Subdermal adipose tissue is greatly reduced in *Gpat4*^{-/-} mice. It remains to be determined whether the reductions in adipose tissue are due to attenuated TAG production or to inhibited adipocyte differentiation as occurs with congenital generalized lipodystrophy due to mutations in AGPAT2 [70]. Despite increased energy expenditure and reduced adipose mass and hepatic TAG content, *Gpat4*^{-/-} mice have no significant differences in fasting plasma glucose or insulin concentrations and can maintain their body temperature when housed at 4 °C for 4 h [68]. *Gpat4* mRNA is also highly expressed in mammary epithelium, where lipids are synthesized for milk production. The content of lipid droplets in mammary epithelial cells is significantly reduced in *Gpat4*^{-/-} mice, suggesting that GPAT4 has an integral role in milk lipid synthesis [67]. Because the content of diacylglycerol and TAG in milk is reduced approximately 90%, most pups born to *Gpat4*^{-/-} mice die within the first 48 hours after birth, unless nursed by a foster mother.

Overexpression of GPAT4 does not increase TAG production in either HEK293 cells [11] or Cos-7 cells, despite a 60% increase in NEM-sensitive GPAT activity [10]. Because livers from *Gpat4*^{-/-} mice contain 45% less TAG than livers from wildtype mice, we assume that GPAT4 *in vivo* normally initiates the synthesis of TAG [68] and that the cultured cells lacked some cellular machinery required to enable the esterification of the excess DAG synthesized under these conditions [10]. However, DAG synthesized via GPAT1 in the same cultured cells was able to increase incorporation of [¹⁴C]oleic acid into both diacylglycerol and TAG pools [10,44]. Overexpression of GPAT4 in cultured cells increased [¹⁴C]oleic acid incorporation into phosphatidylinositol [10], but overexpression of the highly homologous GPAT3 in HEK293 cells increased [¹⁴C]oleic acid incorporation only into TAG and not phospholipids [71]. These data suggest that the individual GPAT isoforms may generate distinct lipid intermediates destined for specific biosynthetic pathways. [10].

What is the relationship of the GPAT isoforms to the formation of lipid droplets?

We are presently faced with a plethora of GPAT isoforms; all four isoforms are apparently able to initiate the glycerolipid synthetic pathway and at least three of these isoforms are able to increase the incorporation of fatty acid into TAG. Although overexpression of GPAT4 in cultured cells curiously did not increase TAG synthesis, the diminished hepatic TAG content in *Gpat4*^{-/-} mice suggests that its LPA product, too, can be incorporated into TAG.

GPAT has always been considered to be the rate-limiting step in the pathway of glycerolipid synthesis and to regulate fatty acid flux through the pathway (Figure 2). It seems probable that the biosynthesis of the two major lipid components of lipid droplets, the phospholipid monolayer and the core TAG, must be synchronized. Thus, it is likely that the different GPAT isoforms work in a coordinated way to synthesize these lipids. In that sense, it is puzzling that a marked increase in TAG synthesis is not accompanied by enhanced synthesis of phospholipids, primarily phosphatidylcholine, required to coat the surfaces of newly forming lipid droplets [72]. Further, growing evidence indicates that the lipid intermediates in the glycerolipid synthetic pathway may initiate intracellular signaling via protein kinase C and similar pathways [46,49]. It is likely that the regulation of lipid droplet associated proteins is also inherent to lipid synthesis. Much work remains to fully delineate the transcriptional and acute regulation of each GPAT isoform as well as its individual role in the synthesis of TAG, specific phospholipids, and lipid intermediates involved in signaling.

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References

1. Kennedy EP. Biosynthesis of complex lipids. *Fed Proc* 1961;20:934–940. [PubMed: 14455159]
2. Weiss SB, Kennedy EP. The enzymatic synthesis of triglycerides. *J Am Chem Soc* 1956;78:3550.
3. Monroy G, Kelker HC, Pullman ME. Partial purification and properties of an acyl coenzyme A:*sn*-glycerol 3-phosphate acyltransferase from rat liver mitochondria. *J Biol Chem* 1973;248:2845–2852.
4. Coleman RA, Lee DP. Enzymes of triacylglycerol synthesis and their regulation. *Prog Lipid Res* 2004;43:134–176. [PubMed: 14654091]
5. Yet SF, Lee S, Hahn YT, Sul HS. Expression and identification of p90 as the murine mitochondrial glycerol-3-phosphate acyltransferase. *Biochemistry* 1993;32:9486–9491. [PubMed: 8369314]
6. Bhat BG, Wang P, Kim JH, Black TM, Lewin TM, Fiedorek TF, Coleman RA. Rat hepatic *sn*-glycerol-3-phosphate acyltransferase: Molecular cloning and characterization of the cDNA and expressed protein. *Biochim Biophys Acta* 1999;1439:415–423. [PubMed: 10446428]
7. Wang S, Lee DP, Gong N, Schwerbrock NMJ, Mashek DG, Gonzalez-Baró MR, Stapleton CM, Li LO, Lewin TM, Coleman RA. Cloning and functional characterization of a novel mitochondrial *N*-ethylmaleimide-sensitive glycerol-3-phosphate acyltransferase (GPAT2). *Arch Biochem Biophys* 2007;465:347–358. [PubMed: 17689486]
8. Harada N, Hara S, Yoshida M, Zenitani T, Mawatari K, Nakano M, Takahashi A, Hosaka T, Yoshimoto K, Nakaya Y. Molecular cloning of a murine glycerol-3-phosphate acyltransferase-like protein 1 (xGPAT1). *Mol Cell Biochem* 2007;297:41–51. [PubMed: 17013544]
9. Cao J, Li JL, Li D, Tobin JF, Gimeno RE. Molecular identification of microsomal acyl-CoA:glycerol-3-phosphate acyltransferase, a key enzyme in de novo triacylglycerol synthesis. *Proc Natl Acad Sci USA* 2006;103:19695–19700. [PubMed: 17170135]
10. Nagle CA, Verges L, Wang S, deJong H, Wang S, Lewin TM, Reue K, Coleman RA. Identification of a novel *sn*-glycerol-3-phosphate acyltransferase isoform, GPAT4 as the enzyme deficient in *Atp6b*^{-/-} mice. *J Lipid Res*. 2008
11. Chen Y, Kuo MS, Li S, Bui HH, Peake DA, Sanders PE, Thibodeaux SJ, Chu S, Qian YW, Zhao Y, Bredt DS, Moller DE, Konrad RJ, Eigneux AP, Young SG, Cao G. AGPAT6 Is a novel microsomal glycerol-3-phosphate acyltransferase (GPAT). *J Biol Chem*. 2008
12. Lewin TM, Schwerbrock NMJ, Lee DP, Coleman RA. Identification of a new glycerol-3-phosphate acyltransferase isoenzyme, mtGPAT2, in mitochondria. *J Biol Chem* 2004;279:13488–13495. [PubMed: 14724270]
13. Neuwald AF. Barth syndrome may be due to an acyltransferase deficiency. *Current Biol* 1997;7:R465–R466.

14. Heath RJ, Rock CO. A missense mutation accounts for the defect in the glycerol-3-phosphate acyltransferase expressed in the *plsB26* mutant. *J Bacteriol* 1999;181:1944–1946. [PubMed: 10074094]
15. Lewin TM, Wang P, Coleman RA. Analysis of amino acid motifs diagnostic for the *sn*-glycerol-3-phosphate acyltransferase reaction. *Biochemistry* 1999;38:5764–5771. [PubMed: 10231527]
16. Dircks LK, Ke J, Sul HS. A conserved seven amino acid stretch important for murine mitochondrial glycerol-3-phosphate acyltransferase activity: significance of arginine 318 in catalysis. *J Biol Chem* 1999;274:34728–34734. [PubMed: 10574940]
17. Ofman R, Hettema EH, Hogenhout EM, Caruso U, Muijsers AO, Wanders RJA. Acyl-CoA:dihydroxyacetonephosphate acyltransferase: cloning of the human cDNA and resolution of the molecular basis in rhizomelic chondrodysplasia punctata type 2. *Hum Mol Genet* 1998;7:847–853. [PubMed: 9536089]
18. Heath RJ, Rock CO. A conserved histidine is essential for glycerolipid acyltransferase catalysis. *J Bacteriol* 1998;180:1425–1430. [PubMed: 9515909]
19. Soupene E, Fyrst H, Kuypers FA. Mammalian acyl-CoA:lysophosphatidylcholine acyltransferase enzymes. *Proc Natl Acad Sci USA* 2008;105:88–93. [PubMed: 18156367]
20. Zhao Y, Chen Y, Bonacci TM, Brecht DS, Li S, Bensh WR, Moller DE, Kowala M, Konrad RJ, Cao G. Identification and characterization of a lysophosphatidylcholine acyltransferase that is primarily expressed in metabolic tissues. *J Biol Chem*. 2008
21. Cao Y, Pearman AT, Zimmerman GA, McIntyre TM, Prescott SM. Intracellular unesterified arachidonic acid signals apoptosis. *PNAS* 2000;97:11280–11285. [PubMed: 11005842]
22. Cao J, Liu Y, Lockwood J, Burn P, Shi Y. A novel cardiolipin-remodeling pathway revealed by a gene encoding an endoplasmic reticulum-associated Acyl-CoA:lysocardiolipin acyltransferase (ALCAT1) in mouse. *J Biol Chem* 2004;279:31727–31734. [PubMed: 15152008]
23. Lu B, Jiang YJ, Zhou YT, Xu FY, Hatch GM, Choy PC. Cloning and characterization of murine 1-acyl-*sn*-glycerol-3-phosphate acyltransferases and their regulation by PPAR α in murine heart. *Biochem J* 2004;385:469–77. [PubMed: 15367102]
24. Nakanishi H, Shindou H, Hishikawa D, Harayama T, Ogasawara R, Suwabe A, Taguchi R, Shimizu T. Cloning and characterization of mouse lung-type acyl-CoA:lysophosphatidylcholine acyltransferase 1 (LPCAT1). Expression in alveolar type II cells and possible involvement in surfactant production. *J Biol Chem* 2006;281:20140–20147. [PubMed: 16704971]
25. Yang Y, Cao J, Shi Y. Identification and characterization of a gene encoding human LPGAT1, an endoplasmic reticulum-associated lysophosphatidylglycerol acyltransferase. *J Biol Chem* 2004;279:55866–55874. [PubMed: 15485873]
26. Xu Y, Kelley RI, Blanck TJ, Schlame M. Remodeling of cardiolipin by phospholipid transacylation. *J Biol Chem* 2003;278:51380–51385. [PubMed: 14551214]
27. Xu Y, Malhotra A, Ren M, Schlame M. The enzymatic function of tafazzin. *J Biol Chem* 2006;281:39217–39224. [PubMed: 17082194]
28. Testet E, Laroche-Traineau J, Noubhani A, Coulon D, Bunoust O, Camougrand N, Manon S, Lessire R, Bessoule JJ. Ypr140wp, ‘the yeast tafazzin’, displays a mitochondrial lysophosphatidylcholine (lyso-PC) acyltransferase activity related to triacylglycerol and mitochondrial lipid synthesis. *Biochem J* 2005;387:617–626. [PubMed: 15588229]
29. Agarwal AK, Arioglu E, De Almeida S, Akkoc N, Taylor SI, Bowcock AM, Barnes RI, Garg A. AGPAT2 is mutated in congenital generalized lipodystrophy linked to chromosome 9q34. *Nat Genet* 2002;31:21–23. [PubMed: 11967537]
30. Johnston JM, Kelley RI, Feigenbaum A, Cox GF, Iyer GS, Funanage VL, Proujansky R. Mutation characterization and genotype-phenotype correlation in Barth syndrome. *Am J Hum Genet* 1997;61:1053–1058. [PubMed: 9345098]
31. Dircks LK, Sul HS. Mammalian mitochondrial glycerol-3-phosphate acyltransferase. *Biochim Biophys Acta* 1997;1348:17–26. [PubMed: 9370312]
32. Lewin TM, Granger DA, Kim JH, Coleman RA. Regulation of mitochondrial *sn*-glycerol-3-phosphate acyltransferase activity: response to feeding status is unique in various rat tissues and is discordant with protein expression. *Arch Biochem Biophys* 2001;396:119–127. [PubMed: 11716470]

33. Bell, RM.; Coleman, RA. The Enzymes. Boyer, PD., editor. Academic Press; New York: 1983. p. 87-112.
34. Shin DH, Paulauskis JD, Moustaid N, Sul HS. Transcriptional regulation of p90 with sequence homology to *Escherichia coli* glycerol-3-phosphate acyltransferase. J Biol Chem 1991;266:23834-23839. [PubMed: 1721057]
35. Horton JD. Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis. Biochem Soc Trans 2002;30:1091-1095. [PubMed: 12440980]
36. Eberle D, Hegarty BD, Bossard P, Ferre P, Foufelle F. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 2004;86:839-848. [PubMed: 15589694]
37. Jerkins AA, Liu WR, Lee S, Sul HS. Characterization of the murine mitochondrial glycerol-3-phosphate acyltransferase promoter. J Biol Chem 1995;270:1416-1421. [PubMed: 7836409]
38. Ericsson J, Jackson SM, Kim JB, Spiegelman BM, Edwards PA. Identification of glycerol-3-phosphate acyltransferase as an adipocyte determination and differentiation factor 1-and sterol regulatory element-binding protein-responsive gene. J Biol Chem 1997;272:7298-7305. [PubMed: 9054427]
39. Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K, Gotoda T, Ishibashi S, Yamada N. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 1999;274:35832-35839. [PubMed: 10585467]
40. Yoshikawa T, Shimano H, Amemiya-Kudo M, Yahagi N, Hasty AH, Matsuzaka T, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Kimura S, Ishibashi S, Yamada N. Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Mol Cell Biol 2001;21:2991-3000. [PubMed: 11287605]
41. Cha JY, Repa JJ. The liver X receptor (LXR) and hepatic lipogenesis: The carbohydrate-response element binding protein is a target gene of LXR. J Biol Chem 2007;282:743-751. [PubMed: 17107947]
42. Park H, Kaushik VK, Constant S, Prentki M, Przybytkowski E, Ruderman NB, Saha AK. Coordinate regulation of malonyl-CoA decarboxylase, *sn*-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. J Biol Chem 2002;277:32571-32577. [PubMed: 12065578]
43. Darnley AC, Carpenter CA, Saggerson ED. Changes in activities of some enzymes of glycerolipid synthesis in brown adipose tissue of cold-acclimated rats. Biochem J 1988;253:351-355. [PubMed: 3178717]
44. Igal RA, Wang S, Gonzalez-Baró M, Coleman RA. Mitochondrial glycerol phosphate acyltransferase directs incorporation of exogenous fatty acids into triacylglycerol. J Biol Chem 2001;276:42205-42212. [PubMed: 11546763]
45. Lewin TM, Wang S, Nagle CA, Van Horn CG, Coleman RA. Mitochondrial glycerol-3-phosphate acyltransferase-1 directs the metabolic fate of exogenous fatty acids in hepatocytes. Am J Physiol Endocrinol Metab 2005;288:E835-E844. [PubMed: 15598672]
46. Nagle CA, An J, Shiota M, Torres TP, Cline GW, Liu ZX, Wang S, Catlin RL, Shulman GI, Newgard CB, Coleman RA. Hepatic overexpression of glycerol-sn-3-phosphate acyltransferase 1 in rats causes insulin resistance. J Biol Chem 2007;282:14807-14815. [PubMed: 17389595]
47. Hammond LE, Gallagher PA, Wang S, Posey-Marcos E, Hiller S, Kluckman K, Maeda N, Coleman RA. Mitochondrial glycerol-3-phosphate acyltransferase-deficient mice have reduced weight and liver triacylglycerol content and altered glycerolipid fatty acid composition. Mol Cell Biol 2002;22:8204-8214. [PubMed: 12417724]
48. Hammond LE, Neschen S, Romanelli AJ, Cline GW, Ilkayeva OR, Shulman GI, Muoio DM, Coleman RA. Mitochondrial glycerol-3-phosphate acyltransferase-1 is essential in liver for the metabolism of excess acyl-CoAs. J Biol Chem 2005;280:25629-25636. [PubMed: 15878874]
49. Neschen S, Morino K, Hammond LE, Zhang D, Liu ZX, Romanelli AJ, Cline GW, Pongratz RL, Zhang XM, Choi CS, Coleman RA, Shulman GI. Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1 knock out mice. Cell Metab 2005;2:55-65. [PubMed: 16054099]

50. Xu H, Wilcox D, Nguyen P, Voorbach M, Suhar T, Morgan SJ, An WF, Ge L, Green J, Wu Z, Gimeno RE, Reilly R, Jacobson PB, Collins CA, Landschulz K, Surowy T. Hepatic knockdown of mitochondrial GPAT1 in ob/ob mice improves metabolic profile. *Biochem Biophys Res Commun* 2006;349:439–448. [PubMed: 16935266]
51. Winder WW, Thomson DM. Cellular energy sensing and signaling by AMP-activated protein kinase. *Cell Biochem Biophys* 2007;47:332–347. [PubMed: 17652779]
52. Muoio DM, Dohm GL, Tapscott EB, Coleman RA. Leptin opposes insulin's effects on fatty acid partitioning in muscles isolated from obese ob/ob mice. *Am J Physiol* 1999;276:E913–E921. [PubMed: 10329986]
53. Onorato TM, Chakraborty S, Haldar D. Phosphorylation of rat liver mitochondrial glycerol-3-phosphate acyltransferase by casein kinase 2. *J Biol Chem* 2005;280:19527–19534. [PubMed: 15778226]
54. Collison LW, Kannan L, Onorato TM, Knudsen J, Haldar D, Jolly CA. Aging reduces glycerol-3-phosphate acyltransferase activity in activated rat splenic T-lymphocytes. *Biochim Biophys Acta* 2005;1687:164–172. [PubMed: 15708364]
55. Kerner J, Distler AM, Minkler P, Parland W, Peterman SM, Hoppel CL. Phosphorylation of rat liver mitochondrial carnitine palmitoyltransferase-I: effect on the kinetic properties of the enzyme. *J Biol Chem* 2004;279:41104–41113. [PubMed: 15247243]
56. Coleman RA, Lewin TM, Muoio DM. Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. *Ann Rev Nutr* 2000;20:77–103. [PubMed: 10940327]
57. Coleman RA, Haynes EB. Selective changes in microsomal enzymes of triacylglycerol and phosphatidylcholine synthesis in fetal and postnatal rat liver: Induction of microsomal *sn*-glycerol 3-P and dihydroxyacetone-P acyltransferase activities. *J Biol Chem* 1983;258:450–465. [PubMed: 6848513]
58. Coleman RA, Bell RM. Selective changes in enzymes of the *sn*-glycerol-3-phosphate and dihydroxyacetone-phosphate pathways of triacylglycerol biosynthesis during differentiation of 3T3-L1 pre-adipocytes. *J Biol Chem* 1980;255:7681–7687. [PubMed: 6156941]
59. Muoio DM, Seefield K, Witters L, Coleman RA. AMP-activated kinase (AMPK) reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: Evidence that *sn*-glycerol-3-phosphate acyltransferase is novel target. *Biochem J* 1999;338:783–791. [PubMed: 10051453]
60. Bates EJ, Topping DL, Sooranna SP, Saggerson DE, Mayes PA. Acute effects of insulin on glycerolphosphate acyltransferase activity, ketogenesis and serum free fatty acid concentration in perfused rat liver. *FEBS Lett* 1977;84:225–228. [PubMed: 598501]
61. Bates EJ, Saggerson ED. A study of the glycerol phosphate acyltransferase and dihydroxyacetone phosphate acyltransferase activities in rat liver mitochondrial and microsomal fractions. Relative distribution in parenchymal and non-parenchymal cells, effects of N-ethylmaleimide, palmitoyl-coenzyme A concentration, starvation, adrenalectomy and anti-insulin serum treatment. *Biochem J* 1979;182:751–762. [PubMed: 518562]
62. Farese RV, Standaert ML, Yamada K, Huang LC, Zhang C, Cooper DR, Wang Z, Yang Y, Suzuki S, Toyota T, Lerner J. Insulin-induced activation of glycerol-3-phosphate acyltransferase by a chiro-inositol-containing insulin mediator is defective in adipocytes of insulin-resistant, type II diabetic, Goto-Kakizaki rats. *Proc Natl Acad Sci USA* 1994;91:11040–11044. [PubMed: 7972005]
63. Vila MC, Farese RV. Insulin rapidly increases glycerol-3-phosphate acyltransferase activity in rat adipocytes. *Arch Biochem Biophys* 1991;284:366–368. [PubMed: 1846521]
64. Vila MC, Milligan G, Standaert ML, Farese RV. Insulin activates glycerol-3-phosphate acyltransferase (*de novo* phosphatidic acid synthesis) through a phospholipid-derived mediator. Apparent involvement of $G_{i\alpha}$ and activation of phospholipase C. *Biochemistry* 1990;29:8735–8740. [PubMed: 2176832]
65. Yamada K, Standaert ML, Yu B, Mischak H, Cooper DR, Farese RV. Insulin-like effects of sodium orthovanadate on diacylglycerol-protein kinase C signaling in BC3H-1 myocytes. *Arch Biochem Biophys* 1994;312:167–172. [PubMed: 8031124]

66. Tang W, Yuan J, Chen X, Gu X, Luo K, Li J, Wan B, Wang Y, Yu L. Identification of a novel human lysophosphatidic acid acyltransferase, LPAAT-theta, which activates mTOR pathway. *J Biochem Mol Biol* 2006;39:626–635. [PubMed: 17002884]
67. Beigneux AP, Vergnes L, Qiao X, Quatela S, Davis RG, Watkins SM, Coleman RA, Walzem RL, Philips M, Reue K, Young SG. Agpat6--a novel lipid biosynthetic gene required for triacylglycerol production in mammary epithelium. *J Lipid Res* 2006;47:734–744. [PubMed: 16449762]
68. Vergnes L, Beigneux AP, Davis RG, Watkins SM, Young SG, Reue K. Agpat6 deficiency causes subdermal lipodystrophy and resistance to obesity. *J Lipid Res* 2006;47:745–754. [PubMed: 16436371]
69. Li D, Yu L, Wu H, Shan Y, Guo J, Dang Y, Wei Y, Zhao S. Cloning and identification of the human LPAAT-zeta gene, a novel member of the lysophosphatidic acid acyltransferase family. *J Hum Genet* 2003;48:438–442. [PubMed: 12938015]
70. Gale SE, Frolov A, Han X, Bickel PE, Cao L, Bowcock AM, Schaffer JE, Ory DS. A regulatory role for 1-acylglycerol-3-phosphate-O-acyltransferase 2 in adipocyte differentiation. *J Biol Chem* 2006;281:11082–11089. [PubMed: 16495223]
71. Cao J, Burn P, Shi Y. Properties of the mouse intestinal acyl- CoA:monoacylglycerol acyltransferase, MGAT2. *J Biol Chem*. 2003Ms. 302835200
72. Tauchi-Sato K, Ozeki S, Houjou T, Taguchi R, Fujimoto T. The surface of lipid droplets is a phospholipid monolayer with a unique fatty acid composition. *J Biol Chem* 2002;277:44507–44512. [PubMed: 12221100]
73. Gonzalez-Baró MR, Granger DA, Coleman RA. Mitochondrial glycerol phosphate acyltransferase contains two transmembrane domains with the active site in the N-terminus, facing the cytosol. *J Biol Chem* 2001;276:43182–43188. [PubMed: 11557771]

Figure 1.

Domain structure of GPAT1–4. The black regions represent the active site with the four active site motifs in yellow. Putative transmembrane domains (TMDs) are shown as blue squares. Only the TMDs and topography of GPAT1 have been confirmed experimentally; its N- and C-termini face the cytosol and the loop between the TMDs faces the mitochondrial intramembrane space [73].

Figure 2.

Multiple isoforms catalyze each step in the glycerol-3-phosphate pathway of TAG synthesis. GPAT1 and GPAT2 on the outer mitochondrial membrane compete with carnitine palmitoyl transferase-1 for acyl-CoAs that may be used for glycerolipid biosynthesis or for β -oxidation. Like GPAT1 and 2, GPAT3 and GPAT4 catalyze the esterification of glycerol-3-phosphate to form lysophosphatidic acid (LPA). As many as 5 possible 1-acyl-*sn*-glycerol-3-phosphate acyltransferases (AGPAT; also known as LPAAT) esterify LPA to form phosphatidic acid (PA). Three isoforms of phosphatidic acid phosphohydrolase (PAPase, which is also known as lipin), hydrolyze the phosphate to form diacylglycerol (DAG). Two major isoforms of diacylglycerol acyltransferase (DGAT) catalyze the third esterification to form triacylglycerol (TAG). Both PA and DAG are also precursors for the glycerophospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), and cardiolipin (CL). The DAG produced by this pathway can activate protein kinase C.