AGPAT6 Is a Novel Microsomal Glycerol-3-Phosphate Acyltransferase (GPAT)*

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Running title: Identification of a novel microsomal GPAT

AGPAT6 is a member of 1-Acylglycerol-3-phosphate O-acyltransferase (AGPAT) family that appears to be important in triglyceride (TG) biosynthesis in several tissues, but the precise biochemical function of the enzyme is unknown. In the current study, we show that AGPAT6 is a microsomal glycerol-3-phosphate (G3P) acyltransferase (GPAT). Membranes from HEK 293 cells overexpressing human AGPAT6 had higher levels of GPAT activity. Substrate specificity studies suggested that AGPAT6 was active against both saturated and unsaturated long-chain fatty acyl-CoAs. Both G3P and fatty acyl-CoA increased the GPAT activity, and the activity was sensitive to N-ethylmaleimide (NEM), a sulfhydryl modifying reagent. Purified AGPAT6 protein possessed GPAT activity but not AGPAT activity. Using [13C7]oleic acid labeling and mass spectroscopy, we found that overexpression of AGPAT6 increased both lysophosphatidic acid (LPA) and phosphatidic acid (PA) levels in cells. In these studies, total TG and phosphatidylcholine (PC) levels were not significantly altered, although there were significant changes in the abundance of specific PC species. Human AGPAT6 is localized to endoplasmic reticulum and is broadly distributed in tissues. Membranes of mammary epithelial cells from Agpat6-deficient mice exhibited markedly reduced GPAT activity compared with membranes from wild-type mice. Reducing AGPAT6 expression in HEK293 cells through siRNA knockdown suggested that AGPAT6 significantly contributed to HEK293 cellular GPAT activity. Our data indicate that AGPAT6 is a microsomal GPAT, and we propose renaming this enzyme GPAT4.

The main glycerolipid biosynthesis pathway uses glycerol-3-phosphate (G3P) and fatty acyl-CoA as substrates to produce lysophosphatidic acid (LPA). This reaction is catalyzed by G3P acyltransferases (GPATs). LPA is then converted to phosphatidic acid (PA), which is catalyzed by 1-acylglycerol-3-phosphate O-acyltransferase (AGPAT, also named LPAAT for LPA acyl-CoA acyltransferase). Dephosphorylation of PA by PA phosphatase (PAP) generates diacylglycerol (DG), which serves as the substrate for diacylglycerol acyltransferase (DGAT) in the formation of triglycerides (TGs). PA and DG can also enter the phospholipid (PL) biosynthetic pathways, leading to the formation of a variety of PL species.

GPAT is regarded as the rate-limiting enzyme for the G3P biosynthetic pathway (1). In yeast, two genes (GAT1 and GAT2) encode two separate GPAT proteins that share amino acid sequence homology with mammalian mitochondria GPAT (2). Plants have three GPATs, one membrane bound and the other two are located within cytosol and chloroplasts (3). Two GPAT activities exist in most mammalian cells and tissues. One resides in the microsomal fraction, and the other is located within mitochondria (4). The enzyme responsible for mitochondrial GPAT activity was purified and the cDNA was cloned (5,6). Mitochondria GPAT (named GPAT1) contains two transmembrane helices and is located in the outer membrane of mitochondria (7). GPAT1 appears to prefer
saturated fatty acyl-CoAs as substrates, and its enzymatic activity is resistant to sulfhydryl modifying agents such as N-ethylmaleimide (NEM) (4). Overexpression of GPAT1 in primary hepatocytes or in the liver leads to increased TG synthesis and reduced fatty acid oxidation (8,9). Conversely, mice deficient in GPAT1 exhibit reduced fat mass, lower body weight, reduced hepatic TG content, and improved insulin sensitivity (10,11). Because residual GPAT activities were observed in mitochondria of Gpat1-deficient mice, it was hypothesized that a second mitochondrial GPAT enzyme must exist (12). That enzyme was recently identified and designated GPAT2 (13).

Mitochondria GPAT activity comprises only 30–50% of total GPAT activity in the liver and about 10% of GPAT activity in most other tissues, suggesting that microsomal GPAT is a major contributor to total GPAT activity (4). The identity of the microsomal GPAT has been elusive, primarily due to the difficulty in purifying the enzyme. Recently, Cao et al. (14) reported the cloning of a microsomal GPAT (named GPAT3) that is induced during adipocyte differentiation. The expression of GPAT3, while high in adipose tissue, is fairly low in other tissues, suggesting the existence of other GPATs (14).

Two AGPAT enzymes were identified in recent years, followed by the identification of an entire family of putative AGPAT proteins that share amino acid sequence similarities (15,16). The biochemical roles for most of these proteins, however, are not clear. AGPAT8 was recently identified as GPAT3 (14). Several recent reports indicated that AGPAT9 was a lysophosphatidylcholine acyltransferase (LPCAT1) (17,18). AGPAT6 is one member of this “AGPAT family,” and its potential physiological role was recently studied using Agpat6-deficient mice (19-21). AGPAT6 is expressed in brown adipose tissue, mammary epithelium, as well as many other tissues. A deficiency of AGPAT6 in mice results in resistance to diet-induced and genetic forms of obesity. Brown fat mass was significantly reduced, and DG and TG levels in milk and subdermal fat were reduced by nearly 90% (20,21). These studies suggest that AGPAT6 has a significant role in lipid biosynthesis. However, the precise biochemical function of AGPAT6 remains unknown. In this study, we identified AGPAT6 as a novel microsomal GPAT and renamed it GPAT4.

**EXPERIMENTAL PROCEDURES**

**Cloning and Expression of AGPAT6**—Full-length human AGPAT6 coding sequence (NCBI accession number NM_178819) was amplified by PCR amplification from human Ovary Marathon Ready cDNA (BD bioscience and Clontech, Palo Alto, CA) and the sequence was subsequently confirmed. A FLAG epitope was inserted in-frame at the carboxyl terminus of the protein by PCR-based cloning. The cDNA was digested at HindIII and XbaI sites and subcloned into the mammalian expression vector p3xFLAG-CMV-14 (Sigma, St Louis, MO). The human GPAT1 cDNA was obtained by PCR amplification from a human full-length cDNA clone TC106982 (Orgene Technologies, Rockville, MD). A FLAG epitope was inserted in-frame at the carboxyl terminus of the protein by PCR-based cloning. The PCR-amplified segment was digested with EcoRI and BamHI sites and subcloned into the mammalian expression vector p3xFLAG-CMV-14. The human GPAT3 was obtained similarly by PCR amplification of a human Heart Marathon Ready cDNA library (Clontech) followed by restriction digestion with HindIII and BamHI sites and subcloning into the mammalian expression vector p3xFLAG-CMV-14. A FLAG epitope was inserted in-frame at the carboxyl terminus of the protein by PCR-based cloning. The human DGAT1 clone was generated as described (22). HEK293 cells were transiently transfected by using Fugene6 (Roche Diagnostics, Indianapolis, IN). The cells were washed with PBS and homogenized with three short 10-sec pulses from a Brinkmann Polytron in 20 mM Tris-HCl, pH 7.4, 250 mM sucrose, 1 mM EDTA and a Protease inhibitor cocktail (Roche Diagnostics). Total membrane fractions (100,000 x g pellet) were resuspended in a homogenization buffer and frozen at −80°C until use. Protein concentrations were determined with a Protein Assay Kit (Pierce, Rockford, IL) with BSA as a standard. Expression of FLAG-tagged AGPAT6, GPAT1, and GPAT3 was verified by immunoblot analysis with anti-Flag M2 antibody (Sigma).

**Purification of AGPAT6**—Two liters of HEK293E suspension cells were transfected with plasmids encoding a Flag-tagged human AGPAT6. The cells were harvested at 42 hours post-transfection and homogenized with five short 30-sec pulses...
from Brinkmann Polytron in 30 ml buffer A (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 250 mM sucrose) containing a protease inhibitor cocktail (Roche) and centrifuged at 600 x g for 5 min to remove large debris and nuclei. Mitochondria fraction was removed by centrifuging the supernatant at 10,300 x g for 10 min. The microsomal fraction was acquired by centrifuging the supernatant for 1 hr at 100,000 x g and solublized in buffer B (20 mM Tris-HCL, pH 7.5, 1 mM EDTA, 250 mM sucrose, 150 mM NaCl, 0.2% Tween 20) on ice for 1 hour. AGPAT6 in the soluble fraction was purified by anti-Flag M2 affinity chromatography and eluted with buffer B containing 0.3 mg/ml 3X FLAG peptide according to the manufacture’s instructions (Sigma). The identity of the purified protein was confirmed by anti-Flag M2 immunoblot analysis and the peptide sequencing.

**siRNA Transfection** — 200 pmol of human GPAM siRNA (siRNA ID number 112114; Ambion, Austin, TX), human GPAT4 siRNA (siRNA ID number J-010300-11; Dharmacon, Lafayette, CO) and control siRNA (silencer negative control 1; Ambion) were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. The RNAs of the transfected cells were prepared for reverse transcription and real-time PCR 2 days after the siRNA transfection. 5 µg of membrane proteins from the transfected cells were used for the in vitro GPAT activity assay.

**In Vitro Acyltransferase Assay**—GPAT activity was determined in a final volume of 100 µl as previously described with some modifications (12,14). The assay was conducted in 75 mM Tris-HCl, pH 7.5, 4 mM MgCl2, 1 mM DTT, 4 mM NaF, 1 mg/ml fatty acid–free BSA with 80 µM [14C]G3P (55 mCi/mmol) (American Radiolabeled Chemicals, St. Louis, MO) and 20 µM fatty acyl-CoA [lauroyl-CoA (C12:0), palmitoyl-CoA (C16:0), stearoyl-CoA (C18:0), oleoyl-CoA (C18:1), linoleoyl-CoA (C18:2), and arachidonoyl-CoA (C20:4) (Sigma)] or 20 µM [14C]fatty acyl-CoA ([14C]lauroyl-CoA (55 mCi/mmol) or [14C]palmitoyl-CoA (50 mCi/mmol)) and 200 µM G3P as substrates. The reaction was initiated by adding 5 µg of membrane proteins. All assays were performed for 20 min at room temperature unless indicated. Lipids were extracted with chloroform: methanol (2:1, vol/vol), dried, and separated by TLC with chloroform:methanol:water (65:25:4) followed by exposure to a phosphor imager screen. Where indicated, membrane proteins were incubated with or without 0.1, 0.5, 50 µM NEM for 15 min on ice before the initiation of reaction. Acyltransferase activity with LPA, lysophosphatidylcholine, lysophosphatidylserine, lysophosphatidylglycerol, lysophosphatidylethanolamine, and DAG substrates were assayed with 20 µM [14C]oleoyl-CoA (55 mCi/mmol) and 200-µM acyl acceptors as described (14). All experiments were performed at least three times.

**Metabolic Labeling Studies**—HEK293 cells (40 h after transfection) were incubated with 2 µM [14C]oleic acid (54 mCi/mmol) in medium supplemented with 0.2% fatty acid–free BSA for 6 or 20 h. Cells were washed twice with cold PBS, and total lipids were extracted with chloroform:methanol (2:1). Lipids were resolved by TLC with chloroform:methanol:water (65:25:4, polar lipids) or hexane:ethyl ethanol:acetic acid (80:20:1, neutral lipids) and visualized with a phosphor imager. For metabolic labeling followed by LC/MS studies, 40 h after transfection, HEK293 cells were incubated with 30 µM (1,2,3,7,8,9,10)-[13C7]oleic acid (Sigma) in medium supplemented with 1% fatty acid–free BSA for 3 or 6 h. Cells were then washed twice with cold PBS, and total lipids were extracted and analyzed by TLC.

**LPA and PA Measurement by LC-MS**—Acyl-lysophosphatic acids (LPAs; LPA C14:0, LPA C16:0, LPA C17:0, LPA C18:1, and LPA C18:0) and diacylphosphatic acids (PA C16:0, C16:0, PA C17:0, C17:0, PA C18:0, C18:0, PA C16:0, C18:1, PA C18:0, C18:1, and PA C18:1, C18:1) were purchased from Avanti Polar Lipids (Alabaster, AL). Stock standard solutions of each of the LPAs, PAs, and internal standards (LPA C17:0, and PA C17:0, C17:0, 50 ng/ml) were prepared in MeOH:Water (80:20) containing 0.1% DEA. Calibration standard solutions were prepared at concentrations ranging from 5 to 250 ng/ml. The high-performance liquid chromatography (HPLC) system consisted of an Agilent 1100 binary pump (Palo Alto, CA), a CTC Leap Technologies HTS PAL Autosampler (Carbboro, NC) equipped with a 100-µl syringe, a Valco C6WK injector (Houston, TX), and a 10-µl sample loop. The analytical column was an
The HPLC mobile phases consisted of methanol:chloroform:water (55:5:40) with 0.1% DEA (A) and methanol:chloroform (50:50) with 0.1% DEA (B). A gradient program was set for the separation at a flow rate of 1 ml/min. The initial mobile phase was 100%, then linearly changed to 80% of B at 0.5 min, then slowly changed to 100% of B at 0.2 min, maintained for 0.8 min, followed by re-equilibration to initial conditions for 0.5 min. Each run time was 2 min. The HPLC system was coupled on-line to a TSQ Quantum triple quadrupole mass spectrometer (Waters, MA, USA). Electrospray ionization (ESI) was performed in the negative mode with an ionspray voltage of 3.3 kV. Cone gas flow and desolvation gas flow were 37 and 697 L/h, respectively. The heated nebulizer temperature was set at 300°C and the source temperature was 130°C. The mass spectrometer operated with LM1, HM1, LM2, and HM2 resolution at 13.5. Multiple reaction monitoring (MRM) detection for LPA and PA was used for quantitation with a dwell time of 20 msec for each transition.

**PC Measurement by LC/MS**—Cell pellets were suspended in 1 ml of methanol, and 200 µl was removed for ceramide analysis. The remaining 800 µl was spiked with 5 nmol C14:0, C16:0PC, 5 nmol C21:0, C21:0PC, 5 nmol C17:0SM, 10 nmol d5-Tripalmitin, and 10 nmol d5-Tristearin internal standards prior to extraction. 1.0 ml of chloroform was added to the 800-µl aliquot of cell pellets, and the sample was vortexed prior to transferring to a 4-ml screw cap vial. Water (400 µl) was added to the extract, and the sample was vortexed and centrifuged 10 min at 4000 rpm. The lower layer was carefully removed. 50 µl of 5 N HCl and 1 ml of chloroform were added to the remaining aqueous layer and the extraction process was repeated. The lower chloroform layers from both extractions were combined and evaporated in a second 4-ml vial. The lipids were reconstituted in 1.0 ml of methanol:chloroform (1:1). A 500-µl aliquot was evaporated and reconstituted in 500 µl of methanol:chloroform (3:1) with 10 mM ammonium acetate. 10 µl of the sample was analyzed in duplicate for PC/SM. After the PC/SM analysis, the sample was evaporated and reconstituted in 200 µl of methanol:chloroform (3:1) with 10 mM ammonium acetate for TG analysis. Phosphatidylcholine (PC) levels were measured via a flow-injection ESI-MS/MS method, adapted from the method of Schmitz and coworkers (23), suitable for rapid monitoring of PC and SM at µmol/l to mmol/l levels. Protonated molecular ions of PC/SM species were selected by precursor ion scans of m/z 184—the fragment ion containing the charged phosphatidylcholine head-group. The ion intensities across the flow-injection profile were summed, and after isotope correction, the quantities of each PC/SM species were calculated relative to PC and SM internal standards.

**TG Analysis by LC-MS**—TG species containing C18:0, C18:1, and 13C7-C18:1 fatty acids were identified by flow-injection ESI and MS/MS scans for losses of the neutral loss (NL) of fatty acid and ammonia from the (M+NH4)+ molecular ions (24,25). The amount of each TG was calculated from analyte to internal standard ratios and the concentration of d5-(C18:0)3 TG internal standard (Avanti Polar Lipids). For TG species analysis, a 1.0-µl aliquot of the various cell extracts were in a mixture of isopropanol:methanol:chloroform (4:2:1). The sample was vortexed and centrifuged, and then transferred to the nano-infusion device. Cells were extracted in the presence of isotopically labeled internal standards of TG. Data were acquired on an LTQ FT equipped with a 7 tesla magnet (Thermo Electron Corporation, San Jose, CA). Electrospray (ESI) was accomplished with the Advin (Ithaca, NY) Triversa Nanomate. The 4.2-micron chip was held at 1.3 kV with 0.2 psi backpressure of nitrogen. Product ion scans were acquired with a collision value of 28% with no ramping of the values. The heated capillary was held at about 150°C. Xcalibur version 2.0 for the LTQ FT was used to generate the scan functions in these experiments. Data were acquired with the LTQ portion of the hybrid. The scan function was accomplished with the inclusion list feature, which commanded the mass spectrometer to generate product ions in a sequential fashion, starting at m/z 250 through m/z 1000. Each product ion event consists of two scans, each scan consisting of two separate scan events. Thus, in the roughly 1.2 seconds of acquisition, 4 scans were acquired for each precursor mass. A single acquisition results in 750 individual product ion events. This approach allowed the use of native quantitation software to produce ratios with the internal standards. This was accomplished using peak heights. Thus, for this study, the product ion of d5 C16 TG internal standard was chosen as the
reference peak (m/z 556.7). This ion was used to normalize the TG product ions that either contained the [13C7]oleic acid side chain or the normal unlabeled product ions.

**Human AGPAT6 Subcellular Localization**—COS-7 cells were grown and transfected with Flag-tagged human AGPAT6 on glass-bottom culture dishes (P35GC, MetTek Corporation, Ashland, MA). To achieve mitochondrial staining, cells were incubated with 100 nM MitoTracker Orange CMTMRos (Invitrogen) for 10 min at 37°C. The cells were then washed twice with PBS and fixed with 4.0% paraformaldehyde prewarmed for 20 min at 37°C. The samples were rinsed twice with PBS and permeabilized with 0.2% Triton X-100 in PBS. The samples were then incubated for 2 h at room temperature with mouse monoclonal anti-FLAG M2 antibody (5.0 µg/ml, Sigma) or rabbit anti-calnexin N-terminal polyclonal antibody (1.0 µg/ml, StressGen Biotechnologies Corp, Victoria, Canada) in PBS with 1% of BSA. After briefly washing with PBS, the samples were incubated for 1 h at room temperature with Alexa Fluor 488 Goat Anti-Mouse SFX or Alexa Fluor 555 Goat Anti-Rabbit SFX (Invitrogen). The cells were counterstained with Propidium Iodide Nucleic Acid Stain (Invitrogen) in PBS. The samples were analyzed with a confocal fluorescence microscope (Olympus BX61, Nashua, NH).

**Quantitative PCR Analysis of Human AGPAT6**—Human normal tissue cDNA panels were obtained from BioChain (Hayward, CA) and PrimGen (Bothell, WA). TaqMan real-time quantitative PCR (Q-PCR) was performed with an ABI Prism 7900 sequence detector (Applied Biosystems, Foster City, CA) with GAPDH as an internal control. Gene-specific primers and probes were obtained from Applied Biosystems. The relative expression of the genes was determined by the Ct method (Applied Biosystems).

**Analysis of Tissue GPAT Activity**—Total membranes from mammary epithelial cells were prepared as follows. The mammary glands were dissected from two-day lactating female mice under ketamine/xylazine anesthesia. Mammary glands were pooled, minced with a razor blade, and digested in 0.2% trypsin/collagenase for 30 min in a 37°C shaker. Adipocytes were eliminated by two rounds of low speed centrifugation (1500 rpm for 10 min), and the remaining pellet containing epithelial cells was treated with AKC buffer (8.3 mg/ml NH₄Cl, 1mg/ml KHCO₃, 36 µg/ml EDTA, pH 7.4) to lyse red blood cells. The cell suspension was then filtered through a 250-μm nylon mesh. Epithelial cells were pelleted by centrifugation at 1500 rpm, and resuspended in homogenization buffer (10 mM Tris, pH 7.4, 1 mM EDTA, 250 mM sucrose, 50 mM NaF, 30 mM Na₃P₂O₇, 100 µM Na₃VO₄, 1 mM DTT). Cells were disrupted by sonication, and the cell lysate was cleared by low speed centrifugation (500 × g for 10 min at 4°C). Total membrane fractions were obtained by ultracentrifugation (90,000 rpm for 1 h at 4°C) and then resuspended in 10 mM Tris, pH 7.4, 1 mM EDTA, 250 mM sucrose, and 1 mM DTT. 5 µg of membrane proteins from wild-type or Agpat6-deficient mice were used for GPAT activity analyses as described above.

**RESULTS**

**Identification of GPAT Activity for AGPAT6**—To identify potential microsomal GPATs, we searched the databases for proteins containing acyl-CoA glycerolipid acyltransferase motifs. Candidate clones were overexpressed in human embryonic kidney 293 (HEK293) cells. After transfection, membranes were prepared and GPAT activity was assessed with thin layer chromatography (TLC). Membranes from AGPAT6-expressing cells yielded substantial GPAT activity and this enzyme was chosen for further analysis.

**Human AGPAT6 is 456 amino acids in length** and is predicted to contain multiple transmembrane helices (19). Western blots of HEK293 cells transfected with a tagged AGPAT6 expressed a 48-kDa AGPAT protein (Fig. 1A), consistent with the size of mouse AGPAT6 (20). Aside from the 48-kDa protein, a smaller protein was visible in transfected cells, presumably a breakdown product. In labeling experiments involving membranes from transfected cells and either radiolabeled lauroyl-CoA or G3P, we found that AGPAT6 expression yielded GPAT activity (Fig. 1B), as judged by higher levels of LPA and PA (Fig. 1B). The increase of PA levels likely results from endogenous AGPAT activities in HEK293 cells, as no increase in AGPAT activity was conferred by AGPAT6 overexpression (see Fig. 1E, 1F and 2B below). Omitting fatty acyl-CoA from the reaction eliminated LPA and PA synthesis. Increasing either fatty acyl-CoA or G3P substrate concentrations resulted in a...
concentration-dependent increase in LPA and PA products [except with high concentrations of palmitoyl-CoA (≥30 µM) where low levels of activity could have been due to a detergent effect] (Fig. 1C). Microsomal GPAT activity is sensitive to inhibition by sulfhydryl-modifying agents (4) such as N-ethylmaleimide (NEM). As shown in Fig. 1D, the GPAT activity from AGPAT6-expressing cells was inhibited by NEM while mitochondrial GPAT1 activity was minimally affected. To pinpoint the GPAT activity of AGPAT6, we expressed the flag-tagged human AGPAT6 in HEK293 cells and affinity purified the protein (Fig. 1E). The identity of the purified enzyme was confirmed with western blot analysis and the peptide sequencing (data not shown).

To characterize the fatty acyl-CoA substrate specificity, we incubated membranes with different fatty acyl-CoAs (Fig. 2A). GPAT1, as expected, showed activity with C12:0, C16:0, C18:0, as well as C18:1 and C18:2, with some preference for shorter saturated fatty acyl-CoAs. AGPAT6 (GPAT4) was active towards C12:0, C16:0, C18:1, and C18:2, but the activity towards C18:0 was low. To determine if GPAT4 had any additional glycerolipid acyltransferase activities, we used LPA, LPC, LPE, LPS, LPG, or DG as substrates and assessed potential activities by TLC. No other enzymatic activity was observed, suggesting that its GPAT activity was specific (Fig. 2B).

Effects on TG and PC Synthesis When GPAT4 Is Overexpressed in HEK293 Cells—To assess the impact of GPAT4 on TG and PL biosynthetic pathways, we overexpressed GPAT4 in HEK293 cells and then labeled the cells with [13C7]oleic acid. After incubation, the lipids were extracted and subjected to TLC analysis (Fig. 3). Surprisingly, GPAT4 failed to increase TG synthesis at either the 6- or 20-h time points, while both GPAT1 and DGAT1 increased TG synthesis. In these assays, GPAT3 also did not increase TG production (Fig. 3). GPAT4 overexpression did not appear to induce higher levels of phosphatidylcholine (PC) synthesis, although there appeared to be higher levels of phosphatidylethanolamine (PE) (Fig. 3).

To further assess GPAT4 activity in live cells, we overexpressed GPAT4 in HEK293 cells and labeled the cells with [13C7]oleic acid. At 3- or 6-h time points, 13C7-labeled cellular LPA, PA, TG, and PC were analyzed by LC/MS. Total LPA, PA, and PC were also examined. Fig. 4A shows that 13C7-labeled LPA was increased at the 3- and 6-h time points by 3.5- and 4.1-fold, respectively, while total labeled PA was increased by 4.8- and 6.0-fold, respectively. Measurements of total LPA and PA indicated significant increases in both LPA (1.9- and 2.4-fold) and PA (3.2- and 4.0-fold) at the two time points (Fig. 4B). These data confirm the studies with membrane fractions and indicate that AGPAT6 possesses GPAT activity. Differences in 13C7-labeled LPA and PA species are shown in Table 1; differences in total LPA and PA are shown in Tables 2 and 3, respectively.

The 13C7-labeled and total TG and PC levels were analyzed by mass spectrometry. Total labeled TG did not appear to be changed by AGPAT6 (GPAT4) expression (Fig. 5), but there was a slight increase in C50 TGs and a slight reduction in C54 TGs (not shown). The total PC level also did not change (Fig. 6A). However, when PC species were analyzed in detail, we found increases in 13C7-labeled PC 34:1 at 3 and 6 h (p<0.0001), while levels of labeled PC 34:2 and 36:2 PC were reduced (p<0.05) (Fig. 6B). Thus, AGPAT6 (GPAT4) overexpression in HEK293 cells appeared to alter the relative abundance of certain PC species.

Subcellular Localization and the Tissue Distribution of Human GPAT4—Mouse AGPAT6 was shown to be located within the endoplasmic reticulum (20), but differences between mouse and human glycerolipid acyltransferases have been reported (17,18,27). Thus, we performed experiments to define the location of human AGPAT6 (GPAT4). Fig. 7 shows that human AGPAT6 (GPAT4) colocalized with calnexin (an ER marker) while there was no overlapping staining with a mitochondria marker. The tissue distribution of human AGPAT6 (GPAT4) was analyzed by quantitative real-time PCR (Fig. 8A,B). AGPAT6 (GPAT4) was ubiquitously expressed, but there were low expression levels in skeletal muscle.
Reduced GPAT Activity in Mammary Epithelial Membranes from Agpat6 Knockout Mice—The alveoli and ducts of mammary glands of Agpat6 knockout mice had reduced amounts of fat droplets. We compared GPAT activity in membranes from mammary epithelial cells of lactating Agpat6 knockout mice and lactating littermate wild-type mice. As shown in Fig. 9, LPA and PA were decreased by 88% and 64%, respectively, in the Agpat6 knockout mouse mammary gland membranes.

AGPAT6/GPAT4 contributes to HEK293 cellular GPAT activity—To further evaluate the relative contributions to the cellular GPAT activity from AGPAT6/GPAT4, we conducted the siRNA knockdown experiments in HEK293 cells. Membranes from HEK293 cells have considerable GPAT activity. siRNA knockdown of either GPAT4 or mitochondria GPAT1 (GPAM) led to a significant reduction of 72% and 87% in mRNA levels respectively (Fig. 10A). These were associated with a significant 52% and 54% reduction in GPAT activity respectively (Fig. 10B and 10C). These data indicate that AGPAT6/GPAT4 along with mitochondria GPAT are both significant contributors of cellular GPAT activity in HEK293 cells.

DISCUSSION

Our studies identified AGPAT6 as a microsomal GPAT. This GPAT, now designated GPAT4, has broad tissue expression patterns and high levels of expression in adipose tissue and mammary tissue. Membranes from HEK293 cells overexpressing GPAT4 exhibited significantly higher GPAT activity levels with both saturated and unsaturated fatty acid substrates. Moreover, the membranes of Agpat6-deficient mammary epithelial cells exhibited markedly reduced GPAT activity levels. Human GPAT4 is located exclusively in the endoplasmic reticulum. AGPAT6 (GPAT4) activity was sensitive to a sulphydryl-modifying reagent, consistent with known properties of microsomal GPAT (4). Interestingly, GPAT4 contains a cysteine (Cys-325) within a highly conserved domain thought to be important for G3P binding (20).

The mouse orthologue of human GPAT4 (initially designated AGPAT6) was first identified in a gene-trap screen in mouse embryonic stem (ES) cells (20). ES cells containing an insertional mutation in Agpat6 were used to create Agpat6-deficient mice. β-Galactosidase staining studies revealed that the gene was expressed highly in subcutaneous tissue, brown adipose tissue, and mammary epithelium (20). Histological and biochemical studies revealed that Agpat6-deficient mice had lower amounts of subcutaneous adipose tissue, reduced triglyceride levels in mammary epithelial cells and maternal milk, and reduced triglyceride levels in brown adipose tissue. In those earlier studies (20), biochemical experiments failed to identify any AGPAT enzymatic activity for AGPAT6—consistent with our current findings. The identification of AGPAT6 in the mouse gene-trap screen prompted efforts to identify additional members of the AGPAT family and to define evolutionary relationships between different AGPAT enzymes. These studies uncovered a number of new “AGPAT” family members including one, initially designated AGPAT8, that was very closely related to AGPAT6 (66% identical at the amino acid level) (20). AGPAT8 was recently shown to have GPAT activity and was renamed GPAT3 (14). In addition to the identification of AGPAT6 as a GPAT, we also convincingly ruled out any AGPAT activities through two experiments. First, using membranes from HEK293 cells overexpressing GPAT4/AGPAT6, addition of LPA and fatty acyl-CoA did not lead to increased PA formation compared to the vector transfection control. Secondly, using affinity purified AGPAT6/GPAT4 proteins, we found no AGPAT activity while GPAT activity was demonstrated. These data are consistent with the original report that no AGPAT activity could be detected when AGPAT6 was overexpressed (20).

Although expression of GPAT4 in cultured cells increased the amount of LPA and PA, it did not increase total TG biosynthesis. This result was surprising, for several reasons. First, expression of mitochondrial GPAT1 or the newly identified mitochondrial GPAT2 increases TG synthesis in cultured cells (14,28). Second, GPAT3 has been reported by others to increase triglyceride synthesis (14). Third, the reduced amounts of triglyceride in certain tissues of Agpat6-deficient mice, for example mammary epithelium, suggest that AGPAT6 could be important for TG production. At this point, we do not understand why GPAT4 overexpression did not yield a clear increase in TG synthesis.
Our studies involved traditional biochemical experiments with radiolabeled substrates, followed by the identification of reaction products by thin-layer chromatography. These studies showed that GPAT4 overexpression yields higher levels of LPA and PA. In addition, we incubated cells with an oleic acid substrate containing $^{13}$C and took advantage of mass spectrometry to measure reaction products. Once again, we found higher levels of LPA and PA, but not TGs or PLs. The mass spectrometry studies did uncover increased levels of $^{13}$C$_7$-labeled 34:1 phospholipids and reduced levels of 34:2 and 36:2 phospholipids, as well as minor changes in the incorporation of $^{13}$C-labeled oleic acid into certain triglyceride species. Although these compositional differences were significant and reproducible, their significance is not clear. We do not know whether these changes are the direct result of increases in GPAT4 reaction products (i.e., the direct result of GPAT4 substrate preferences and subsequent channeling of GPAT4 reaction products) or a very indirect consequence of changes in GPAT4 substrate and product concentrations within the cell. In any case, the mass spectroscopy experiments produced unequivocal evidence to show that AGPAT6 is a bona fide GPAT enzyme.

In summary, metabolic labeling and mass spectroscopy showed that overexpression of AGPAT6 (GPAT4) results in higher levels of LPA and PA production, and the absence of AGPAT6 (GPAT4) results in lower levels of LPA and PA production. AGPAT6 (GPAT4) is located in the endoplasmic reticulum and is sensitive to NEM. Our studies identify AGPAT6 as a microsomal GPAT, and we propose renaming this enzyme GPAT4.
References

FOOTNOTES

*We thank John Lockwood and Dr. Craig Hammond for help in metabolic labeling studies. We thank Patricia Solenberg, Eric Su, Timothy Ryan and June Onyia for insightful discussions. We are indebted to Richard Higgs for help with statistical analyses. This work was supported by BayGenomics, a Program for Genomics Applications from the National Heart, Lung, and Blood Institute (UO1 HL66621).

1The abbreviations used are: G3P, glycerol-3-phosphate AGPAT, 1-acylglycerol-3-phosphate O-acyltransferase; GPAT, glycerol-3-phosphate acyltransferase; PA, phosphatidic acid; LPA, lysophosphatidic acid, TG, triglycerides; PC, phophatidylcholine; PL, phospholipids; LC/MS, liquid chromatography/mass spectroscopy
FIGURE LEGENDS

FIG. 1. **Identification and characterization of GPAT activity.** *A,* Expression of human AGPAT6 (GPAT4) and GPAT1 expression in HEK293 cells. GPAT4 or GPAT1 was expressed with a C-terminal FLAG tag in HEK293 cells. Cell extracts were resolved in SDS-PAGE followed by western blot analysis with anti-FLAG antibody. *B,* GPAT activity analyzed by TLC with either [14C]glycerol-3-phosphate (G3P, Left) or [14C]lauroyl-CoA (Right). Membranes were prepared from control HEK293 cells or cells overexpressing AGPAT6 (GPAT4) and the GPAT assay was carried out as described in the *Experimental Procedures.* Numbers represent the relative levels of radiolabeled LPA and PA products. Ori, origin of migration; FFA, free fatty acid. *C,* Dependence of GPAT activity on substrate concentration. GPAT assays were conducted with HEK293 cell membranes at the indicated concentrations of G3P or palmitoyl-CoA in the presence of 20 μM [14C]palmitoyl-CoA or [14C]G3P, respectively. Representative TLC images indicating the formation of LPA and PA are shown on the left, and the quantitative assessment of relative GPAT activities are shown on the right. *D,* GPAT activity conferred by GPAT4 is sensitive to NEM. Enzymatic activity was analyzed with the membrane proteins from HEK293 cells transfected with vector, GPAT4 (AGPAT6), or GPAT1 with increasing concentrations of NEM. Representative TLC image indicating the formation of LPA/PA is shown on left and the quantitative assessment of GPAT activity sensitivity to NEM inhibition is on the right. *E,* Flag tag affinity-purified human AGPAT6. Human AGPAT6 was expressed in HEK293 cells and purified as described in Methods. Purified Flag-tagged AGPAT6 was analyzed by SDS-PAGE and stained with SimplyBlue SafeStain. *F,* Purified AGPAT6 has GPAT activity, but no AGPAT activity. GPAT activity was assessed using different volumes of proteins from total microsomal, soluble and elute fractions as described in the Methods. The respective protein concentrations of total, soluble and elute fractions were 3.6, 2.9 and 0.5 mg/ml.

FIG. 2. **Substrate specificity of GPAT4.** *A,* GPAT activity with different fatty acyl-CoA species as substrates. Lauroyl-CoA (C12:0), palmitoyl-CoA (C16:0), stearoyl-CoA (C18:0), oleoyl-CoA (C18:1), linoleoyl-CoA (C18:2), and arachidonoyl-CoA (C20:4) were used as acyl-donors, and GPAT assay was performed as in Fig. 1B. Data are representative of two independent experiments. *B,* GPAT4 did not have enzymatic activities of other glycerolipid acyltransferases. Different glycerolipids were used as acyl acceptors to examine any other potential glycerolipid acyltransferase activities for AGPAT6. PA, phosphatidic acid; PG, phosphatidylglycerol; PS, phosphatidylserine; PC, phosphatidylcholine. Data are representative of two independent experiments.
FIG. 3. Overexpression of GPAT4 in mammalian cells did not lead to increased incorporation of [14C]oleic acid into TG. Metabolic labeling studies were performed in HEK293 cells overexpressing human GPAT4, human GPAT3, human DGAT1, or human GPAT1 as described in the Experimental Procedures. On the left, TLC analysis of polar lipids; on the right, TLC analysis of neutral lipids. The number below each band represents band intensity relative to vector control (which was arbitrarily assigned a value of 1). Data were representative of two independent experiments. PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine.

FIG. 4. GPAT activity for AGPAT6 (GPAT4). Lipids from either control HEK293 cells or cells expressing AGPAT6 (GPAT4) (in triplicate) were extracted 3 or 6 h after incubation with a 13C7-labeled oleic acid. LPA and PA levels were analyzed by LC-MS as described in the Experimental Procedures. A, Levels of LPA and PA containing the 13C7-labeled oleic acid. B, Total LPA and PA levels. *p < 0.05.

FIG. 5. Cellular TG synthesis. 13C7-labeled TG levels were analyzed with LC-MS. TGs containing the 13C7-labeled oleic acid label were measured after 3 and 6 h, as described in the Experimental Procedures.

FIG. 6. Cellular PC synthesis. Total PC was analyzed with LC-MS method as described. A, [13C7]Oleic acid–labeled PC and total PC was measured after 3 or 6 h. B, [13C7]Oleic acid incorporation into specific PC species. *p < 0.05; **p < 0.0001.

FIG. 7. Human AGPAT6 is located in the endoplasmic reticulum (ER). COS-7 cells overexpressing Flag-tagged hGPAT4 was visualized by immunofluorescence microscopy with anti-FLAG antibody (A and E). ER and mitochondria were visualized by staining with Alexa Fluor 555 Goat Anti-Rabbit SFX antibody specific for Calnexin (B) and MitoTracker Orange CMTMRos (F), respectively. D and H represented the merged pictures of A with B and E with F, respectively. C and G shows nuclear staining. (Scale bar, 20 µm)

FIG. 8. Tissue distribution of human GPAT4. Quantitative PCR analysis was performed as described in Experimental Procedures using human normal tissue cDNA panel obtained from BioChain (A) or PrimGen (B). Data were expressed as mean ± SD (n = 3).

FIG. 9. Membranes of mammary epithelial cells from AGPAT6-deficient mice exhibit reduced GPAT activity. Membranes from wild-type or AGPAT6-deficient mice were prepared as described in
the Experimental Procedures. GPAT activity was assessed by TLC for both wild-type and AGPAT6-deficient membranes. The amount of LPA and PA with AGPAT6-deficient membranes was quantified and expressed as a percentage of the activity observed with wild-type membranes.

FIG. 10. Mitochondria GPAT (GPAM) and AGPAT6/GPAT4 both contribute to GPAT activities in HEK293 cells. The control siRNA, human GPAM siRNA and human GPAT4 siRNA were transfected into 293 cells. After 48 h, mRNA levels (A) and GPAT activities were measured (B) and quantified (C) as described in experimental procedures. A representative TLC is shown in B. The data in A and C represent the mean ± S.E. of three triplicate measurements. *, p < 0.05.
Table 1. Differences in $^{13}$C$_7$-labeled LPA and PA species between AGPAT6 (GPAT4)-transfected and vector-transfected cells 3 and 6 h after incubating cells with $^{13}$C$_7$-labeled oleic acid.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$[^{13}$C$_7$]LPA (C18:1) (nmoles/mg)</th>
<th>$[^{13}$C$_7$]PA (C16:0, C18:1) (nmoles/mg)</th>
<th>$[^{13}$C$_7$]PA (C18:0, C18:1) (nmoles/mg)</th>
<th>$[^{13}$C$_7$]PA (C18:1, C18:1) (nmoles/mg)</th>
<th>$[^{13}$C$_{14}$]PA (C18:1, C18:1) (nmoles/mg)</th>
<th>Total $[^{13}$C$]LPA$ (nmoles/mg)</th>
<th>Total $[^{13}$C$]PA$ (nmoles/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 h</td>
<td>Vector</td>
<td>0.27 ± 0.03</td>
<td>1.12 ± 0.08</td>
<td>0.26 ± 0.02</td>
<td>0.06 ± 0.05</td>
<td>0.87 ± 0.06</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>AGPAT6</td>
<td>0.97 ± 0.07</td>
<td>7.95 ± 0.60</td>
<td>0.76 ± 0.05</td>
<td>1.33 ± 0.09</td>
<td>3.65 ± 0.30</td>
<td>0.97 ± 0.07</td>
</tr>
<tr>
<td>6 h</td>
<td>Vector</td>
<td>0.35 ± 0.02</td>
<td>1.26 ± 0.09</td>
<td>0.31 ± 0.02</td>
<td>0.78 ± 0.06</td>
<td>0.80 ± 0.06</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>AGPAT6</td>
<td>1.44 ± 0.10</td>
<td>11.7 ± 0.82</td>
<td>0.84 ± 0.06</td>
<td>2.66 ± 0.19</td>
<td>3.77 ± 0.26</td>
<td>1.44 ± 0.10</td>
</tr>
</tbody>
</table>
Table 2. Differences in total LPA species between AGPAT6 (GPAT4)-transfected and vector-transfected cells 3 and 6 h after incubating cells with $^{13}$C$_7$-labeled oleic acid.

<table>
<thead>
<tr>
<th>Sample</th>
<th>LPA (C14:0) (nmoles/mg)</th>
<th>LPA (C16:0) (nmoles/mg)</th>
<th>LPA (C18:1) (nmoles/mg)</th>
<th>LPA (C18:0) (nmoles/mg)</th>
<th>Total LPA (nmoles/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 h Vector</td>
<td>0.144 ± 0.012</td>
<td>0.79 ± 0.06</td>
<td>1.22 ± 0.09</td>
<td>0.437 ± 0.036</td>
<td>2.59 ± 0.12</td>
</tr>
<tr>
<td>AGPAT6</td>
<td>0.295 ± 0.021</td>
<td>2.86 ± 0.20</td>
<td>1.30 ± 0.10</td>
<td>0.838 ± 0.070</td>
<td>5.30 ± 0.24</td>
</tr>
<tr>
<td>6 h Vector</td>
<td>0.161 ± 0.013</td>
<td>0.64 ± 0.06</td>
<td>0.97 ± 0.09</td>
<td>0.392 ± 0.029</td>
<td>2.16 ± 0.11</td>
</tr>
<tr>
<td>AGPAT6</td>
<td>0.313 ± 0.022</td>
<td>2.70 ± 0.19</td>
<td>1.42 ± 0.12</td>
<td>0.855 ± 0.068</td>
<td>5.29 ± 0.24</td>
</tr>
</tbody>
</table>
Table 3. Differences in total PA species between AGPAT6 (GPAT4)-transfected and vector-transfected cells 3 and 6 h after incubating cells with $^{13}$C$_7$-labeled oleic acid.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PA (C16:0, C16:0) (nmoles/mg)</th>
<th>PA (C18:0, C18:0) (nmoles/mg)</th>
<th>PA (C16:0, C18:1) (nmoles/mg)</th>
<th>PA (C18:0, C18:1) (nmoles/mg)</th>
<th>PA (C18:1, C18:1) (nmoles/mg)</th>
<th>Total PA (nmoles/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vector</td>
<td>0.90 ± 0.06</td>
<td>0.128 ± 0.009</td>
<td>4.21 ± 0.29</td>
<td>3.48 ± 0.27</td>
<td>1.99 ± 0.15</td>
<td>10.7 ± 0.43</td>
</tr>
<tr>
<td>AGPAT6</td>
<td>17.4 ± 1.21</td>
<td>0.324 ± 0.025</td>
<td>8.78 ± 0.67</td>
<td>4.51 ± 0.33</td>
<td>2.80 ± 0.23</td>
<td>33.8 ± 1.44</td>
</tr>
<tr>
<td>6 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vector</td>
<td>0.89 ± 0.06</td>
<td>0.107 ± 0.010</td>
<td>2.94 ± 0.28</td>
<td>2.30 ± 0.16</td>
<td>2.05 ± 0.14</td>
<td>8.30 ± 0.36</td>
</tr>
<tr>
<td>AGPAT6</td>
<td>14.3 ± 1.19</td>
<td>0.414 ± 0.033</td>
<td>8.59 ± 0.77</td>
<td>5.67 ± 0.41</td>
<td>3.75 ± 0.30</td>
<td>32.8 ± 1.51</td>
</tr>
</tbody>
</table>
Fig. 1A
Fig. 1B

Lauroyl-CoA + + + – G3P + + + –

78 2.4 1
17 3.8 1
29 3 1

[14C]G3P

G3P + + + –

10 3.8 1
29 3 1

[14C]Lauroyl-CoA

PA
LPA
Or.
FFA
Fig. 1C

- **Vector** and **AGPAT6**

<table>
<thead>
<tr>
<th>G3P (µM)</th>
<th>0</th>
<th>10</th>
<th>30</th>
<th>100</th>
<th>300</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LPA</td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Palmitoyl-CoA (µM)</th>
<th>0</th>
<th>3</th>
<th>10</th>
<th>30</th>
<th>60</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Graphs**

- **LPA + PA (nmol/min/mg)**
  - Vector
  - AGPAT6

- **Palmitoyl-CoA (µM)**
  - Graph showing the effect of Palmitoyl-CoA concentration on LPA + PA production for Vector and AGPAT6 conditions.
Fig. 1D

![Graph showing the effect of NEM on GPAT activity](image)

<table>
<thead>
<tr>
<th>NEM (µM)</th>
<th>Vector</th>
<th>AGPAT6</th>
<th>GPAT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**[14C]G3P and Palmitoyl-CoA**
Fig. 1E

- AGPAT6

kDa
<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Soluble</th>
<th>Elute</th>
</tr>
</thead>
<tbody>
<tr>
<td>µl</td>
<td>10</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.5</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Fig. 1F

[Fig. showing gel analysis of [C^{14}] G3P/Palmitoyl_CoA with bands for PA, LPA, and Ori]
Fig. 2A

12:0 CoA  16:0 CoA  18:0 CoA  18:1 CoA  18:2 CoA

Vector  G PAT1  AGPAT6  Vector  G PAT1  AGPAT6  Vector  G PAT1  AGPAT6  Vector  G PAT1  AGPAT6

[14C]G3P
Fig. 2B

[14C]Oleoyl-CoA
Neutral lipids
(hexane:ethyl ether:acetic acid)
(80:20:1)

Polar lipids
(chloroform:methanol:water)
(65:25:4)

Fig. 3
TG levels (nmoles/mg protein)

- Vector: 9.50, 10.0, 14.63
- AGPAT6: 10.45, 17.71, 14.63

Time points:
- 3 h
- 6 h
Fig. 6B

Bar graph showing PC levels (nmol/mg protein) for different conditions.

- **[13C<sub>7</sub>]PC (34:1)**
- **[13C<sub>7</sub>]PC (34:2)**
- **[13C<sub>7</sub>]PC (36:2)**
- **[13C<sub>14</sub>]PC (36:2)**

Conditions:
- Vector
- AGPAT6

Time points:
- 3 h
- 6 h

Significance levels indicated by asterisks: *p < 0.05, **p < 0.01.
Fig. 7

AGPAT6                   ER  Marker                   Mito Marker

a                          b                              e                          f

AGPAT6                   DNA Merge                   Mito Marker

c                          d                              g                          h
Fig. 8A

AGPAT6 mRNA relative expression

- Skeletal Muscle
- Brain
- Kidney
- Heart
- Liver
- Lung
- Spleen
- Pancreas
- Placenta
Fig. 8B

AGPAT6 mRNA relative expression
Fig. 9

[\textsuperscript{14}C]G3P, Palmitoyl-CoA
Fig. 10

A

Relative mRNA level (%)

Control siRNA  GPAM siRNA  GPAT4 siRNA

B

C

Relative GPAT activity (%)

Control siRNA  GPAM siRNA  GPAT4 siRNA

PA  LPA

*
AGPAT6 Is a novel microsomal glycerol-3-phosphate acyltransferase (GPAT)
YanQun Chen, Ming-Shang Kuo, Shuyu Li, Hai H. Bui, David A. Peake, Philip E. Sanders, Stefan J. Thibodeaux, Shaoyou Chu, Yue-Wei Qian, Yang Zhao, David S. Bredt, David E. Moller, Robert J. Konrad, Anne P. Beigneux, Stephen G. Young and Guoqing Cao

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