

Evidence for regulated monoacylglycerol acyltransferase expression and activity in human liver^S

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Abstract Intrahepatic lipid accumulation is extremely common in obese subjects and is associated with the development of insulin resistance and diabetes. Hepatic diacylglycerol and triacylglycerol synthesis predominantly occurs through acylation of glycerol-3-phosphate. However, an alternative pathway for synthesizing diacylglycerol from monoacylglycerol acyltransferases (MGAT) could also contribute to hepatic glyceride pools. MGAT activity and the expression of the three genes encoding MGAT enzymes (*MOGAT1*, *MOGAT2*, and *MOGAT3*) were determined in liver biopsies from obese human subjects before and after gastric bypass surgery. *MOGAT* expression was also assessed in liver of subjects with nonalcoholic fatty liver disease (NAFLD) or control livers. All *MOGAT* genes were expressed in liver, and hepatic MGAT activity was readily detectable in liver lysates. The hepatic expression of *MOGAT3* was highly correlated with MGAT activity, whereas *MOGAT1* and *MOGAT2* expression was not, and knockdown of *MOGAT3* expression attenuated MGAT activity in a liver-derived cell line. Marked weight loss following gastric bypass surgery was associated with a significant reduction in *MOGAT2* and *MOGAT3* expression, which were also overexpressed in NAFLD subjects. These data suggest that the MGAT pathway is active and dynamically regulated in human liver and could be an important target for pharmacologic intervention for the treatment of obesity-related insulin resistance and NAFLD.—Hall, A. M., K. Kou, Z. Chen, T. A. Pietka, M. Kumar, K. M. Korenblat, K. Lee, K. Ahn, E. Fabbrini, S. Klein, B. Goodwin, and B. N. Finck. Evidence for regulated monoacylglycerol acyltransferase expression and activity in human liver. *J. Lipid Res.* 2012. 53: 990–999.

Supplementary key words hepatic steatosis • triacylglycerol • diacylglycerol • nonalcoholic fatty liver disease

This work was supported by the Pfizer-Washington University Biomedical Research agreement. This work was also supported by a Pilot and Feasibility award from the Washington University Nutrition Obesity Research Center (DK-56341) (to A.M.H.) and by National Institutes of Health Grants DK-078187 (to B.N.F.), DK-56341 and DK-37948 (to S.K.), DK-087821 (to A.M.H.), and UL1-RR-24992 (Washington University Clinical and Translational Science Award). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Manuscript received 14 February 2012 and in revised form 2 March 2012.

Published, JLR Papers in Press, March 6, 2012

DOI 10.1194/jlr.P025536

Obesity is associated with nonalcoholic fatty liver disease (NAFLD), which represents a spectrum of liver abnormalities that are characterized by an increase in intrahepatic triacylglycerol (TAG) content (i.e., steatosis) with or without inflammation and fibrosis (i.e., steatohepatitis). The presence of steatosis is an important marker of metabolic dysfunction and correlates closely with multiorgan insulin resistance and dyslipidemia (1), which are key risk factors for diabetes and coronary artery disease. However, the mechanisms responsible for the link between increased intrahepatic TAG content and metabolic disease are not clear.

It has been hypothesized that the hepatocellular accumulation of specific lipid intermediates, including diacylglycerol (DAG), acyl-CoA, and ceramide, in people with NAFLD is the molecular mechanism driving insulin resistance (2, 3). Accordingly, an understanding of the metabolic interrelationships among hepatic glycerolipid synthesis and lipid intermediates has important physiological and clinical implications. There are two pathways for diacylglycerol synthesis. In the liver, most DAG and TAG are synthesized from the sequential acylation of glycerol-3-phosphate. However, these lipids can also be synthesized from monoacylglycerol through the monoacylglycerol acyltransferase (MGAT) pathway (Fig. 1) (4, 5). The MGAT and glycerol-3-phosphate pathways are convergent and each results in the synthesis of DAG, which is then acylated to form TAG by the DAG acyltransferase (DGAT) enzymes (Fig. 1). The MGAT pathway is an important TAG

Abbreviations: BMI, body mass index; DAG, diacylglycerol; GBS, gastric bypass surgery; HSI, hepatic insulin sensitivity index; HOMA-IR, homeostatic model assessment of insulin resistance; MAPF, methyl arachidonyl fluorophosphonate; MGAT, monoacylglycerol acyltransferase; NAFLD, nonalcoholic fatty liver disease; RYBG, Roux-en-Y gastric bypass; TAG, triacylglycerol; TTR, tracer-to-tracee ratio.

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^SThe online version of this article (available at <http://www.jlr.org>) contains supplementary data in the form of one table.

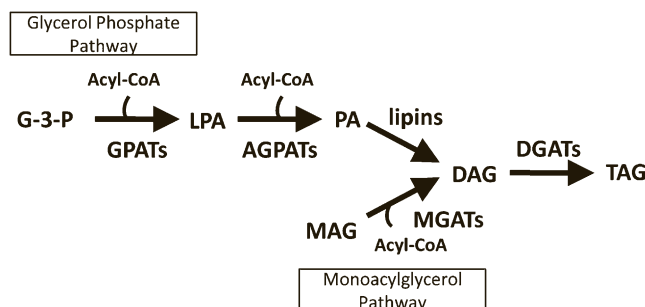


Fig. 1. Two convergent pathways for triacylglycerol biosynthesis. The stepwise acylation of glycerol through the two pathways for triacylglycerol synthesis are shown. AGPAT, acylglycerol-3-phosphate acyltransferase; DAG, diacylglycerol; DGAT, DAG acyltransferase; FA, fatty acid; G-3-P, glycerol-3-phosphate; GPAT, G-3-P acyltransferase; LPA, lysophosphatidic acid; MAG, monoacylglycerol; MGAT, MAG acyltransferase; PA, phosphatidic acid; TAG, triacylglycerol.

biogenesis pathway in intestinal epithelial cells, which re-esterify dietary fat into chylomicron particles. There is evidence that the MGAT pathway is active in neonatal and diabetic rodent liver (6, 7) and may also be present in liver of adult humans (8), but the relevance of MGAT activity to intrahepatic lipid metabolism is unclear.

In the last decade, the genes that encode MGAT activity (9–11), which are known as *MOGATs* to avoid confusion with the genes encoding mannosyl (α -1,3)-glycoprotein β -1,2-N-acetylglucosaminyltransferases, have been cloned. In humans, there are three *MOGAT* family genes (*MOGAT1*, *MOGAT2*, and *MOGAT3*). Rodents also have three *Mogat* genes (*Mogat1*, *Mogat2*, and *Mogat3*). However, the human and murine *MOGAT3/Mogat3* genes are not analogs and evolved separately in postspeciation duplication events. The murine *Mogat3* gene arose from duplication of *Mogat2*, whereas the human *MOGAT3* gene may have arisen from duplication of the gene encoding DGAT2 (12). Human MGAT3 also possesses significant DGAT activity and can catalyze both reactions required to convert monoacylglycerol to TAG (13). In fact, the amino acid sequence of all the MGATs exhibits homology to that of DGAT enzymes, and the two principal DGATs also possess modest MGAT activity (4, 14, 15).

Given the potential implications of MGAT activity in the pathogenesis of hepatic insulin resistance and NAFLD, we sought to evaluate MGAT activity and to characterize the

expression of the *MOGAT* family genes in liver of human subjects. The novel findings of this study are that *i*) the expression of *MOGATs* and MGAT activity is readily detectable in liver of human subjects, *ii*) hepatic MGAT activity is strongly correlated with *MOGAT3* expression in obese human subjects, *iii*) the hepatic expression of *MOGAT2* and *MOGAT3* is significantly downregulated following gastric bypass surgery (GBS)-induced weight loss, and *iv*) hepatic *MOGAT2* and *MOGAT3* expression is increased in subjects with NAFLD. Collectively, these data suggest that the MGAT pathway could be a therapeutic target for pharmacologic intervention for obesity-related hepatic insulin resistance and NAFLD.

RESEARCH DESIGN AND METHODS

Study subjects

In Cohort 1, liver biopsies were obtained from 43 women and 11 men [body mass index (BMI) of 57.4 ± 11.9 kg/m²] during GBS, and *MOGAT* expression and MGAT activity were determined as described below. After subjects fasted overnight (12 h), blood samples were obtained to determine plasma glucose and insulin concentrations. GBS was performed the day after blood samples were collected. Liver tissue was obtained by needle biopsy during the GBS procedure, before gastric stapling and intestinal resection were performed. Hepatic tissue was rinsed in sterile saline, immediately frozen in liquid nitrogen, and then stored at -80°C until subsequent analyses.

Nine subjects from Cohort 1 (Cohort 2; **Table 1**) underwent a stable isotopically labeled glucose tracer infusion the day before GBS and then again one year after surgery to measure endogenous glucose production and the hepatic insulin sensitivity index (HISI). Subjects were admitted to the General Clinical Research Center (GCRC) at Washington University School of Medicine on the evening before the infusion study. At 1800 h, subjects consumed a standard meal, which provided ~ 8 kcal/kg body weight and contained 55% of total energy as carbohydrate, 30% as fat, and 15% as protein. At 0600 h the following morning, a 3 h primed, continuous infusion of $[6,6\text{-}^2\text{H}_2]\text{glucose}$ (priming dose: $22.5 \mu\text{mol}\cdot\text{kg}^{-1}$; infusion rate: $0.25 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), dissolved in 0.9% NaCl solution, was started. Blood samples were obtained before beginning the tracer infusion to determine background plasma glucose tracer-to-tracee ratio (TTR), and every 10 min during the final 30 min of the infusion period to determine insulin concentrations and glucose kinetics. These blood samples were collected in chilled tubes containing sodium EDTA. Samples

TABLE 1. RYGB Cohort 2

	Pre-RYGB (n = 9)	Post-RYGB (n = 9)	P
	Mean \pm SEM (range)	Mean \pm SEM (range)	
Weight (kg)	138.1 \pm 9.4 (104.1–174.2)	85.3 \pm 6.4 (65.3–117.4)	0.0004
BMI (kg/m ²)	51.2 \pm 3.3 (39.5–70.2)	32.0 \pm 2.6 (23.2–47.3)	0.0005
Insulin (mU/l)	18.3 \pm 3.1 (7.4–31.5)	4.7 \pm 0.8 (2.5–9.8)	0.0011
Glucose (mg/dl)	102.9 \pm 4.9 (93.4–136.3)	82.5 \pm 1.7 (75.2–90.0)	0.0019
HOMA-IR score	4.8 \pm 1.0 (2.4–10.6)	0.9 \pm 0.1 (0.5–1.8)	0.0025
HISI	98.8 \pm 18.7 (32–198)	240.8 \pm 30.4 (131–337)	0.0013
TAG (mg/dl)	144 \pm 15.3 (79–220)	72.0 \pm 7.7 (52–126)	0.0006
HDL-cholesterol (mg/dl)	47.1 \pm 3.4 (36–62)	67.6 \pm 4.9 (48–99)	0.0034
LDL-cholesterol (mg/dl)	101.4 \pm 9.9 (64–143)	88.7 \pm 4.8 (73–113)	0.2625
AST (IU/l)	26.3 \pm 5.6 (14–52)	23.6 \pm 4.0 (14–52)	0.7120
ALT (IU/l)	42.2 \pm 19.0 (10–158)	29.8 \pm 6.1 (11–72)	0.5489

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

were placed on ice, and plasma was separated by using refrigerated centrifugation within 30 min of collection and then stored at -80°C until final analyses were performed. In this cohort, liver biopsies were obtained twice, by needle biopsy during GBS and percutaneously one year after surgery during the infusion study.

Cohort 3 (Table 2) was composed of subjects who had a liver biopsy to evaluate elevated serum aminotransferases. Biopsy specimens were divided for histopathologic examination and RNA isolation/gene expression. The diagnosis NAFLD was based on histologic scoring of the liver biopsies. To obtain tissue from non-NAFLD livers, hepatic biopsies from cadaveric donors were obtained at time of transplant.

All subjects gave their written, informed consent before participating in this study, which was approved by the Human Research Protection Office of Washington University School of Medicine, St. Louis, MO.

RNA isolation and quantitative real-time RT-PCR

For tissue profiling studies (Fig. 2), human *MOGAT* gene expression profiling was performed on total RNA purchased from Clontech (Mountain View, CA). Real-time PCR (RT-PCR) was performed using the TaqMan RNA-to- C_T kit (Applied Biosystems, Foster City, CA) and normalized to the expression of peptidylprolyl isomerase A (cyclophilin A, *PPIA*). The sequences of the *MOGAT1* and *PPIA* primer and probes are described in supplementary Table I and were prepared by Integrated DNA Technologies (Coralville, IA). The *MOGAT2* and *MOGAT3* Taqman assays (Hs00228268_m1 and Hs00698325_m1, respectively) were purchased from Applied Biosystems.

For studies presented in Table 3, total RNA was isolated from liver tissues using RNA-Bee (Tel-Test, Friendswood, TX) according to the manufacturer's instructions. Real-time RT-PCR was performed using Applied Biosystems reverse transcription (RT) kit, PowerSYBR PCR mix, and the ABI PRISM 7500 sequence detection system. Arbitrary units of target gene mRNA were corrected to 36B4 RNA content to control for loading. The primers used in these analyses can be found in supplementary Table I.

Sources of human liver and intestine microsome and Western blotting

XTreme 200 human liver microsome and S9 fractions (lot numbers 0810413 and 0810405, respectively) and pooled human intestine microsome and combined cytosol and microsome (S9) fractions (lot numbers 0710352 and 0710351, respectively) were purchased from XenoTech (Lenexa, KS). Membranes overexpressing human MGAT proteins were generated from baculovirus-infected Sf9 cells using standard techniques. For Western blotting, 50 μg of human liver or intestine microsome and S9

fractions were load to 4–12% Novex NuPAGE Bis-Tris precast gels (Invitrogen, Carlsbad, CA). Membranes were blotted in 3% dried milk in TBS-Tween 20 (Sigma-Aldrich, St. Louis, MO) and probed with an MGAT2 or MGAT3 antibody (Santa Cruz Technologies, Santa Cruz, CA) at 4°C overnight.

Isolation of human liver microsomes

Human liver biopsy samples were processed in FastPrep Lysing Matrix tubes (MP Biomedicals, Solon, OH) containing 1 ml of ice-cold microsome preparation buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.25 M sucrose) supplemented with complete, EDTA-free protease inhibitor cocktail according to the manufacturer's instructions (Roche, Indianapolis, IN). The lysate was centrifuged at 400–500 g for 10 min at 4°C , and then the supernatant removed and recentrifuged at 12,000–13,000 g for 10 min at 4°C . The supernatant from the 12,000–13,000 g spin was centrifuged at 104,000–109,000 g for 60 min at 4°C , and the pellet was resuspended in a final volume of 1 ml of microsome preparation buffer and centrifuged under the same conditions. The resultant pellet was resuspended in 20 μl microsome preparation buffer without proteinase inhibitors and protein concentration was determined prior to storage at -80°C .

Determination of MGAT activity

To determine MGAT activity, various amounts of microsomes were incubated with 20 μM [^{14}C]decanoyl-CoA (PerkinElmer, Waltham, MA) and 200 μM 2-oleoylglycerol in 50 mM HEPES, pH 7.4, 10 mM MgCl_2 , 0.001% Triton $\times 100$, 2.5% v/v acetone. A nonselective lipase inhibitor (methyl arachidonoyl fluorophosphonate; MAFP) was added to the reaction to block hydrolysis of both the product and substrate (16). The reaction was incubated at room temperature for 5–60 min and terminated by the addition of 50 μl 1% phosphoric acid. Lipids were extracted with 300 μl of CHCl_3 /methanol (2:1, v/v) and separated by thin-layer chromatography (TLC) with hexane/ethyl ether/acetic acid (80:20:1, v/v/v). The TLC plates were exposed to phosphor screen (GE Healthcare, Piscataway, NJ) and scanned by Phosphor Imager (Molecular Dynamics, Sunnyvale, CA). For determination of MGAT activity in human liver biopsies, a standard curve was generated using the XTreme200 pooled human liver microsomes, and the measured activity was confirmed to be on the linear portion of the standard curve.

Adenoviral-mediated overexpression of human MGAT3

Adenovirus expressing human MGAT3 was generated by ViraQuest (North Liberty, IA). Human hepatocellular carcinoma cells, HepG2, were plated into 96-well plates (40,000/well) and transduced with hMGAT3 adenovirus (MOI = 75) for 48 h. Cells were assayed for lipogenic activity using [^{14}C]glycerol (American Radio Chemicals, St. Louis, MO) (0.2 μCi per well, final

TABLE 2. Cohort 3

	Normal (n = 7) ^a	NAFLD (n = 15) ^b	P
	Mean \pm SEM (range)	Mean \pm SEM (range)	
Age (years)	50 \pm 5 (32–71)	42 \pm 3 (21–53)	0.1008
Weight (kg)	90 \pm 7 (66–111)	97 \pm 6 (72–122)	0.4786
BMI (kg/m^2)	32.2 \pm 4.9 (22.8–44.3)	34.6 \pm 1.7 (24.2–43.3)	0.4679
Insulin (mU/l)	7.7 \pm 2.7 (4.5–13)	23.5 \pm 8. (7.4–125.4)	0.0840
TAG (mg/dl)	90 \pm 230 (58–149)	196 \pm 25 (82–358)	0.0361
HDL-cholesterol (mg/dl)	50 \pm 5 (42–58)	44 \pm 3 (32–67)	0.2978
LDL-cholesterol (mg/dl)	99 \pm 19 (63–127)	126 \pm 8 (73–164)	0.2765
AST (IU/l)	43 \pm 9 (26–76)	67 \pm 10 (13–132)	0.1042
ALT (IU/l)	56 \pm 19 (16–113)	131 \pm 28 (17–352)	0.0375

All subjects tested negative for all strains of hepatitis. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

^aFour males and three females.

^bFour males and ten females.

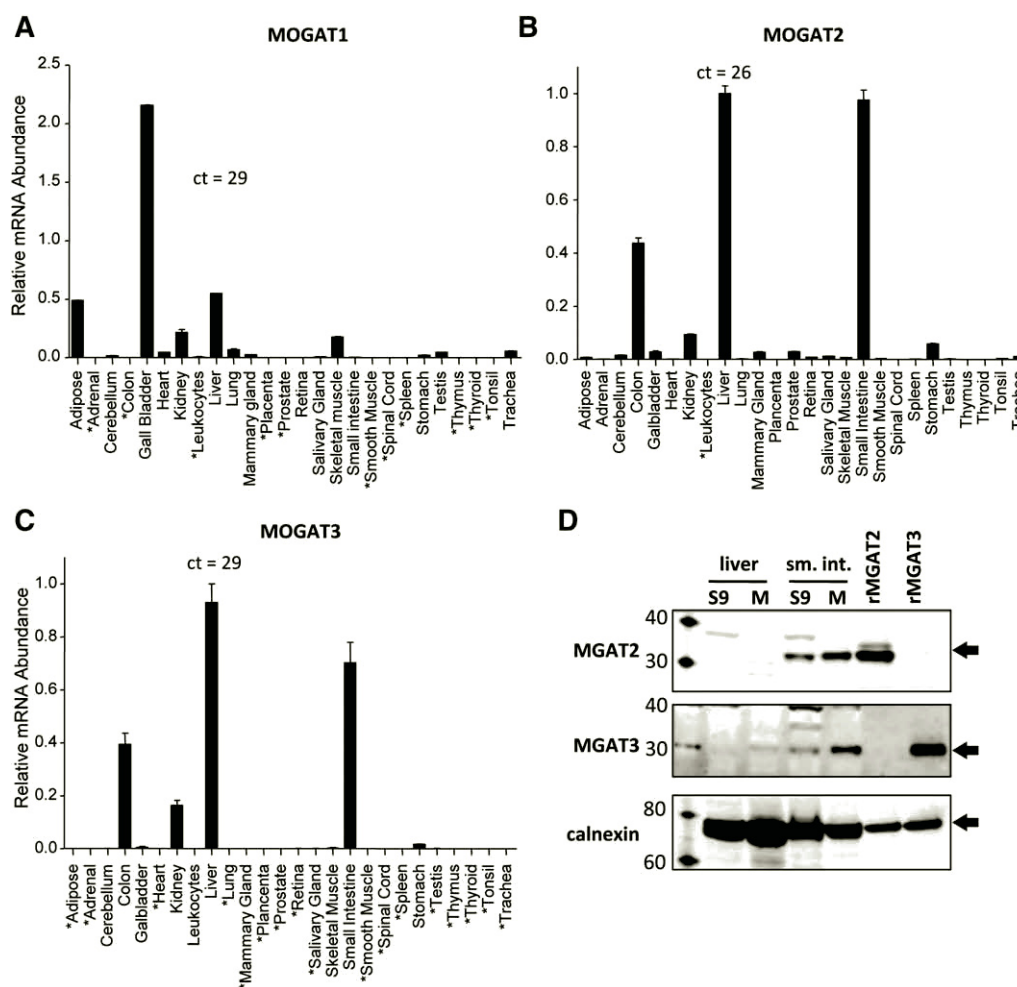


Fig. 2. Tissue-specific expression of human MOGATs. The tissue-specific expression profiles of human *MOGAT1* (A), *MOGAT2* (B), and *MOGAT3* (C) were determined using RT-qPCR. *MOGAT* mRNA levels were normalized to the expression of peptidylprolyl isomerase A (cyclophilin A, *PPIA*). Cycle threshold (CT) in RNA from liver for each gene is shown for reference. Data are mean \pm SD from technical replicates. An asterisk indicates undetectable expression (CT > 35). (D) Western blot analyses for MGAT2 and MGAT3 proteins in S9 and microsomal fractions from human intestine and liver. S9 and microsomal fractions are pools derived from multiple individual donors (n = 200 for liver and n = 18 for intestine). Fifty micrograms of human liver or intestine microsome protein were probed with either MGAT2 or MGAT3 antibodies. Membranes derived with Sf9 cells infected with either human MGAT2 or MGAT3 were used as controls.

concentration 22.6 μ M) in the presence of 400 μ M dodecanoate for 3.5 h. When included, a selective DGAT1 inhibitor (see Example 3 in Ref. 17) was added to a final concentration of 3 μ M in DMSO (0.1% v/v final concentration). Lipids were extracted and analyzed as described above.

siRNA-mediated knockdown of MGATs in human hepatocytes

HepG2 cells were transfected in 24-well plates with either scrambled (Ambion Silencer Select Negative Control No.1; Life

Technologies, Grand Island, NY) or MGAT3-targeting siRNA (Sigma-Aldrich) using SilentFect Lipid Reagent according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA). Following transfection, cells were allowed to recover for 48 h prior to MGAT assay. Cellular MGAT activity was determined as outlined above, except [14 C]mono-oleoyl glycerol (0.5 μ Ci/well, final concentration 18 μ M; American Radio Chemicals) was used as the acyl acceptor to more directly assess MGAT activity. Sodium dodecanoate was added to a final concentration of 400 μ M, and the assay was allowed to proceed for 3.5 h prior to analysis as outlined above.

Sample analyses and calculations

Plasma insulin was determined by a radioimmunoassay, plasma glucose concentrations were measured by using an automated glucose analyzer (Yellow Springs Instruments Inc., Yellow Springs, OH), and plasma glucose TTR was determined by using gas chromatography-mass spectroscopy (GC-MS; MSD 5973 system with capillary column; Hewlett-Packard; Palo Alto, CA), as previously described (18). Isotopic steady-state conditions were achieved

TABLE 3. Correlation coefficients (r) of gene expression to enzyme activity

	DAG	TAG	DAG + TAG
<i>MOGAT1</i>	-0.29	-0.28	-0.29
<i>LPGAT1</i>	0.15	0.18	0.17
<i>DGAT1</i>	0.14	0.14	0.15
<i>DGAT2</i>	0.07	0.10	0.09

$P < 0.01$.

during the final 30 min of the infusion period, so Steele's equation for steady-state conditions was used to calculate glucose kinetics (19). Endogenous glucose production in plasma was calculated by dividing the glucose tracer infusion rate by the average plasma glucose TTR during the last 30 min of the infusion period. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from fasting plasma glucose (in $\mu\text{mol/l}$) and insulin (in mU/l) concentrations. Hepatic insulin sensitivity was determined by the reciprocal of the hepatic insulin resistance index, which was calculated as the product of the basal endogenous glucose production rate (in $\mu\text{mol}\cdot\text{kg}\cdot\text{min}^{-1}$) and fasting plasma insulin concentration (in mU/l) (20).

Statistical analyses

The relationships between tissue gene expression and enzymatic activity were determined by using Pearson's correlation coefficient analyses. Paired *t*-test was used to evaluate the statistical significance of the difference in values before and one year after GBS. Nonparametric Mann-Whitney test was used to evaluate the statistical significance of the difference between normal and NAFLD subjects. A *P* value ≤ 0.05 was considered statistically significant.

RESULTS

MOGAT family gene expression

Human *MOGAT1*, *MOGAT2*, and *MOGAT3* mRNAs were all highly expressed in pooled liver RNA, and *MOGAT2* and *MOGAT3* mRNAs were highly expressed in small intestinal RNA (Fig. 2). In line with earlier observations (21), the human *MOGAT1* transcript was found to be subject to extensive alternative splicing and, surprisingly, and we were unable to amplify the putative full-length *MOGAT1* transcript from reverse-transcribed cDNA from liver, gall bladder, adipose, or intestine (data not shown). Western blot analysis of pooled human intestine (18 pooled donors) and liver samples (200 pooled donors) revealed robust expression of MGAT2 protein in human intestinal S9 and microsomal fraction, whereas MGAT2 protein expression was absent in human liver lysates compared with intestinal lysates (Fig. 2). This finding suggests that MGAT2 content of liver is influenced by posttranscriptional mechanisms because MGAT2 protein content was low relative to *MOGAT2* mRNA levels. In contrast, MGAT3 protein was detected in liver and intestinal extracts (Fig. 2), indicating that this MGAT family protein is expressed at detectable levels in human liver.

MGAT activity in human liver microsomes

We next sought to quantify MGAT activity in human liver. Although MGAT3 can utilize both *sn*-1-monoacylglycerol (MAG) and *sn*-2-MAG as substrate, in the presence of *sn*-1-MAG, it also efficiently functions as a DGAT (11, 13). To specifically examine MGAT activity, *sn*-2-monooleoylglycerol (200 μM) was used as the acyl acceptor and [^{14}C]decanoyl CoA (20 μM) as the acyl donor. Previous work has suggested that MGAT activity is very low in hepatic lysates (22, 23). We postulated that lipase activity, which is reported to be very high in adult liver (24), might be obscuring MGAT activity by depleting the substrate

(MAG) or product (DAG) of MGAT enzymes (25). After profiling several lipase inhibitors (data not shown), it was determined that inclusion of a nonspecific lipase inhibitor (MAFP) in the reaction mixture increased the incorporation of [^{14}C]decanoyl-CoA into DAG and TAG by human liver microsomes (Fig. 3A). In contrast, MAFP had no effect on intestinal MGAT activity. These data suggest that high lipase activity in the liver may mask MGAT activity in hepatic microsomes; henceforth, MAFP was used in all MGAT activity assays.

To directly compare MGAT activity in human liver and intestinal isolates, a microsome titration was performed

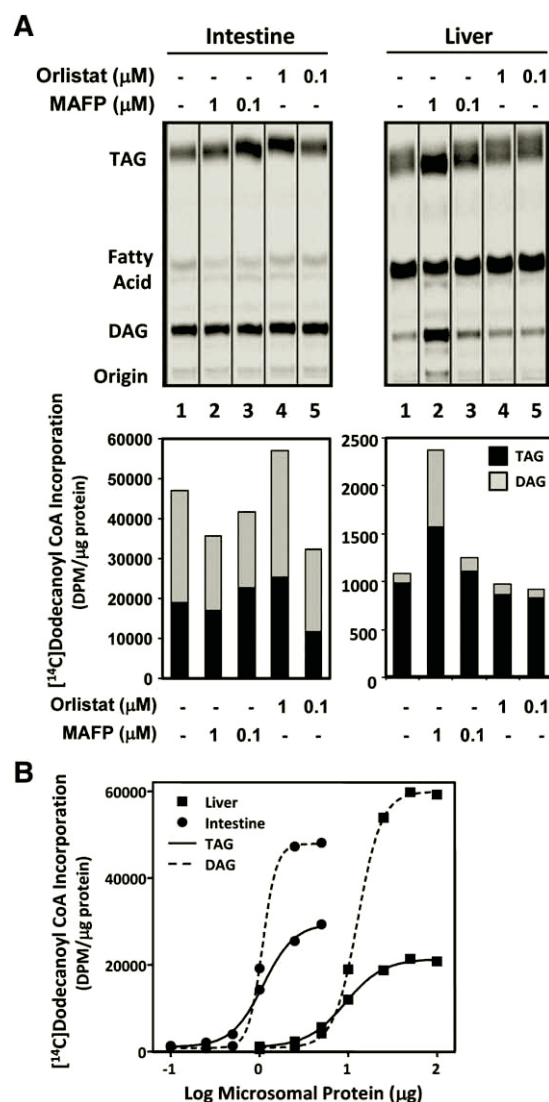


Fig. 3. Expression of human MOGAT2 and MOGAT3 protein and MGAT activity in liver and intestine. (A) The effect of the addition of a nonspecific lipase inhibitor was determined by adding MAFP to reactions containing either pooled human intestinal or liver microsomes and determining the incorporation of [^{14}C]decanoyl-CoA into *sn*-1,2-DAG and TAG. (B). Direct comparison of MGAT activity in human liver (squares) and intestine microsomes (circles). MGAT activity was assessed as previously outlined, and the incorporation of [^{14}C]decanoyl-CoA into TAG and *sn*-1,2-DAG was quantified following TLC. MAFP, methyl arachidonyl fluorophosphonate.

(Fig. 3B). As determined by the production of [14 C] DAG or TAG, approximately 10-fold more liver microsomal protein was required to elicit MGAT activity comparable to that observed in the intestine. Collectively, these data indicate that the overall MGAT activity in human liver, though markedly lower than in intestine, is readily detectable.

MOGAT3 expression is strongly related to hepatic MGAT activity in obese human subjects

MGAT activity of hepatic lysates from 57 obese subjects (Cohort 1) was determined and correlated with the expression of *MOGAT* family genes using parallel biopsies. Prior to assaying the individual human tissue samples, the linearity of the liver microsomal MGAT reaction (20 μ M [14 C]decanoyl-CoA, 200 μ M *sn*-2-monooleoylglycerol) was carefully examined and final assay conditions were determined (Fig. 4). All subsequent MGAT assays were performed at room temperature for 60 min using 5 μ g of microsomal protein.

The expression of *MOGAT3* was strongly related to MGAT activity ($P = 0.01$; Fig. 5) as assessed by incorporation of [14 C]decanoyl CoA into DAG, TAG, or total incorporation into both DAG and TAG. This finding is consistent with MGAT3 acting as both an MGAT and DGAT enzyme (13). In contrast, *MOGAT2* expression was not correlated with MGAT activity (Fig. 5). However, given the disconnect between *MOGAT2* mRNA and MGAT2 protein in liver (Fig. 2), we cannot exclude the possibility that MGAT2 protein levels are regulated independently of the RNA

and thus may correlate with MGAT activity. As expected, *MOGAT1* mRNA levels were not related to MGAT activity (Table 3). Hepatic expression of lysophosphatidylglycerol acyltransferase 1 (*LPGAT1*) and *DGAT* family genes, which also possess MGAT activity in vitro, was not related to MGAT activity (Table 3).

MGAT3 functions as both an MGAT and DGAT enzyme in HepG2 cells

Previous work has suggested that *MOGAT3*, which may have arisen from duplication of the gene encoding DGAT2, encodes an enzyme with both MGAT and DGAT activity. We sought to evaluate this further using a cell-based assay. HepG2 cells were infected with adenovirus expressing MGAT3 and/or GFP, and incorporation of [14 C]glycerol into glycerides was assessed. Overexpression of MGAT3 led to a decrease in the incorporation of [14 C]glycerol into MAG and a modest increase in [14 C]TAG accumulation, but it did not affect [14 C]DAG content of the cells (Fig. 6A). Treatment with a selective DGAT1 inhibitor (DGAT1i; example 3 in Ref. 17) markedly reduced the cellular [14 C]TAG content and caused an increase in [14 C]DAG levels. The effects of the DGAT1i on DAG and TAG were reversed when MGAT3 was overexpressed.

To determine whether loss of MGAT3 affected MGAT activity, HepG2 cells were transfected with siRNA oligonucleotides to knock down *MOGAT3* (Fig. 6B). Loss of MGAT3 by itself did not impair the incorporation of [14 C]mono-oleoyl glycerol into DAG or TAG. However, in the presence of the DGAT1i, the MAG incorporation

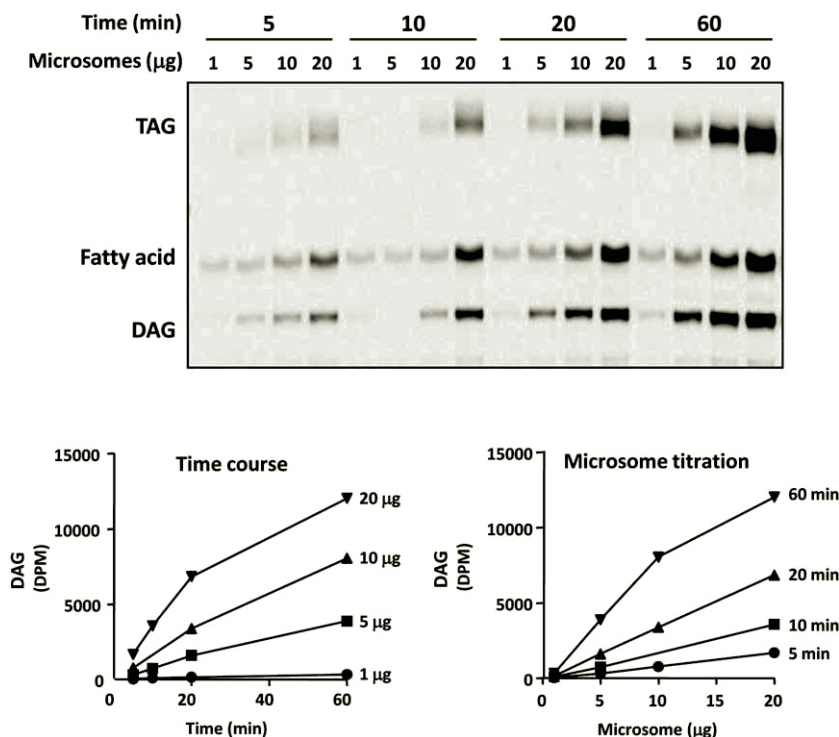


Fig. 4. Characterization of MGAT assay. The effects of time (0–60 min) and protein (1–20 μ g) concentration on MGAT activity were analyzed. MGAT activity was assessed as previously described using [14 C]decanoyl-CoA as the acyl donor and *sn*-2-monooleoylglycerol as the acyl acceptor. Quantification of the *sn*-1,2-DAG is shown below the TLC image.

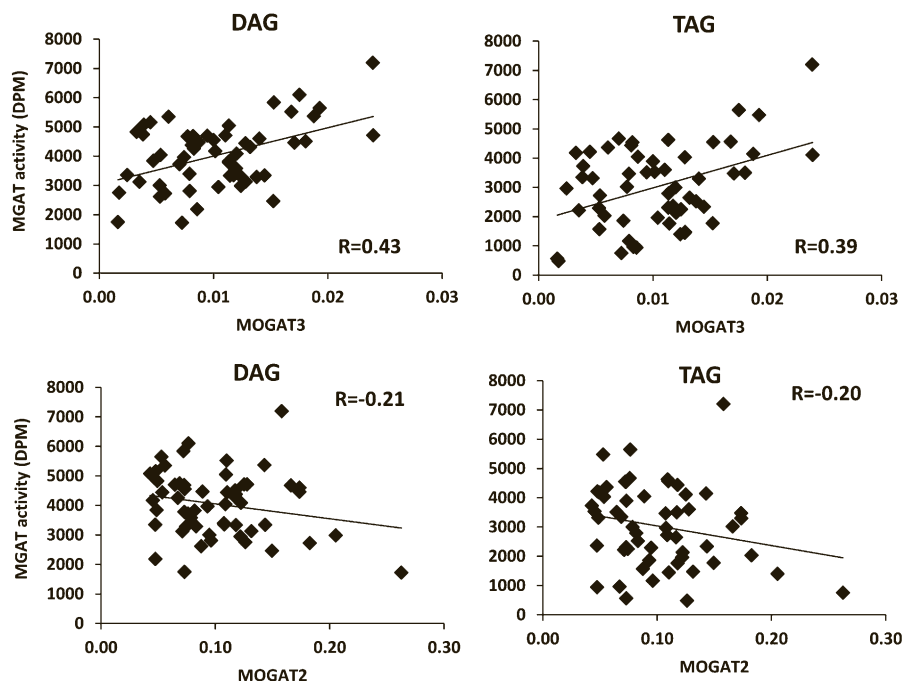


Fig. 5. Hepatic MOGAT3 expression is related to MGAT activity. Scatter plots depict expression of hepatic MOGAT3 and MOGAT2 in relation to hepatic MGAT activity in individual subjects.

into TAG was diminished. Collectively, these data are consistent with the idea that most of the DAG synthesized by MGAT3 is rapidly converted to TAG and that MGAT3 functions as both an MGAT and DGAT enzyme in cultured cells.

MOGAT gene expression is diminished following marked weight loss and is increased in subjects with NAFLD

Finally, we sought to determine whether *MOGAT* expression was regulated in liver by physiologic or pathologic stimuli. *MOGAT2* and *MOGAT3* expression was assessed in nine extremely obese subjects undergoing Roux-en-Y gastric bypass (RYGB; Cohort 2, Table 1). One year after RYGB, subjects had lost 38% of their initial BMI. RYGB led to a significant improvement in systemic and hepatic insulin sensitivity, as measured by the HOMA-IR value and the HISI, and decreased serum TAG concentrations (Table 1). The hepatic expression of *MOGAT2* and *MOGAT3* was significantly downregulated in biopsies obtained one year after RYGB compared with samples obtained during surgery (Fig. 7A).

The expression of *MOGATs* was also assessed in a distinct cohort of subjects with NAFLD (Cohort 3; Table 2). These subjects were obese, but they had an average BMI that was similar to the control group that did not have NAFLD. NAFLD subjects were hypertriglyceridemic ($P < 0.04$), tended to be hyperinsulinemic ($P < 0.08$) and had elevated alanine aminotransferase levels compared with controls (Table 2). *MOGAT2* and *MOGAT3* expression was significantly increased in subjects with NAFLD compared with control subjects (Fig. 7B). Collectively, these data suggest that *MOGAT2* and *MOGAT3* expression is upregulated in subjects with NAFLD and that marked weight loss leads to a correction in the expression of

these glyceride-synthesizing enzymes in extremely obese subjects.

DISCUSSION

An understanding of the factors that control hepatic intermediary glycerolipid metabolism has important implications for the potential treatment of obesity-related metabolic diseases. In this study, we evaluated the expression and activity of the MGAT enzymes in liver of obese human subjects. Our data demonstrate that MGAT activity is present in human liver and that expression of the gene encoding MGAT3 is directly related to hepatic MGAT activity. Furthermore, in a liver-derived cell line, siRNA-mediated knockdown of *MOGAT3* led to reduced MGAT activity. Our data suggest that the expression of the *MOGAT* genes is dynamically regulated in liver of obese human subjects and is increased in subjects with NAFLD. The findings presented here could have significant clinical implications, given the established link between DAG and activation of intracellular signaling pathways that negatively impact hepatic insulin sensitivity (2). Drugs that specifically target MGAT3 could have therapeutic potential for treating hepatic insulin resistance, dyslipidemias, and other metabolic abnormalities associated with hepatic steatosis.

Work conducted in rodent models has suggested that intestinal MGAT activity is important for absorption of dietary fat (26) and that hepatic MGAT activity is important for controlling glycerolipid metabolism in neonatal liver (6, 7). However, data from other studies suggest that the physiological importance of the MGAT enzymes in fatty acid metabolism in adult liver is minimal. For example, some reports show little MGAT activity in hepatic lysates

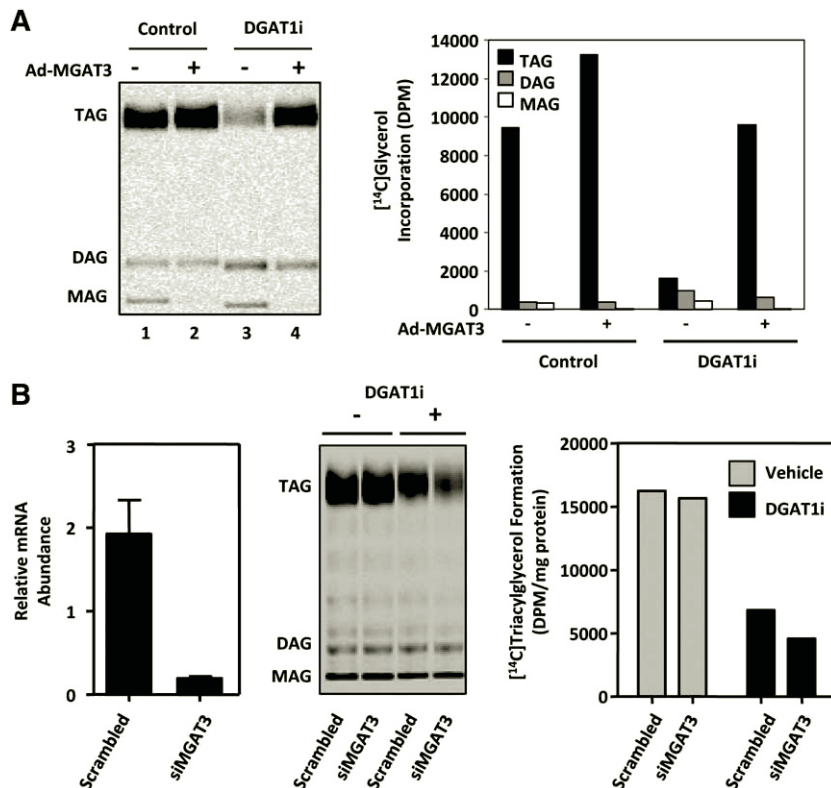


Fig. 6. Cell-based activity of human MGAT3. (A) HepG2 cells were transduced with an adenovirus expressing human MGAT3 (Ad-MGAT3) for 48 h. Cells were assayed for lipogenic activity using [14 C]glycerol in the presence of 400 μ M dodecanoate. A selective DGAT1 inhibitor (DGAT1i) was added to the cells as a 1,000 \times stock in DMSO to a final concentration of 3 μ M in DMSO. Lipids were extracted and analyzed as previously described. A representation of the TLC plate is shown at right. (B) HepG2 cells were transfected with siRNA to MOGAT3 (or scrambled control), and the expression of MOGAT3 (left) or incorporation of [14 C]monooleyl glycerol into TAG (right) was quantified.

from human subjects (23). Similarly, Cortés et al. reported that, despite strong induction of *Mogat1* and *Mogat2* mRNA and MGAT1 protein in the livers of *Agbat2*-deficient mice, they were unable to detect MGAT activity (22). It was postulated this might be due to abundant lipase activity in the liver homogenates assayed. With this in mind, we profiled a number of known lipase inhibitors and found that, in the presence of MAFP, a nonspecific inhibitor of the serine hydrolase superfamily of enzymes which includes multiple lipases, MGAT activity was readily detectable in human liver fractions. The carboxyl ester lipase activity is reported to be high in adult liver (24), and hepatic carboxyl ester lipases are known to be active against both MAG and DAG (25). The inhibition of this lipase may unmask hepatic MGAT activity by preventing the rapid hydrolysis of the MAG substrate and/or newly synthesized DAG or TAG.

The primary metabolic source of MAG, the substrate of MGATs needed for glyceride synthesis in liver, is not known. It is possible that one of the principal functions of MGATs in liver is to recycle MAG derived from lipolysis of intrahepatic TAG and DAG. Recycling of MAG intermediates during periods of rapid TAG turnover may be energetically favorable to new synthesis of glycerol-3-phosphate. Alternatively, the dephosphorylation of lysophosphatidic acid by lipid phosphate phosphatases can directly generate

MAG (27, 28). There is also one report of direct acylation of glycerol (29). The other significant source of MAG in hepatocytes could be derived from hepatic reuptake of lipoprotein/chylomicron particle remnants containing MAG as lipolytic products.

The results from our study underscore the importance of studying human liver tissue to understand the metabolic function and clinical relevance of the MGATs. For example, whereas the rodent *Mogat1* gene encodes a protein that is expressed in stomach and kidney, the human *MOGAT1* gene is primarily noncoding due to extensive alternative splicing (data not shown) (21). The present study also suggests the importance of *MOGAT3*, which is not analogous to murine *Mogat3*, to hepatic MGAT activity. In fact, the mouse *Mogat3* gene is a pseudogene (12). We also detected high expression of *MOGAT* in human liver, which is in close agreement with other reports (8–11) and in contrast to the expression profile in murine species. None of the *Mogat* genes is well expressed in normal mouse liver, suggesting species-specific differences in expression profiles.

Recent work has suggested the potential utility of targeting MGAT activity in treatment of obesity-related diseases of the liver and other organs. It was recently demonstrated that *Mogat2* knockout mice are protected from diet-induced obesity and hepatic steatosis (26). There are also a few

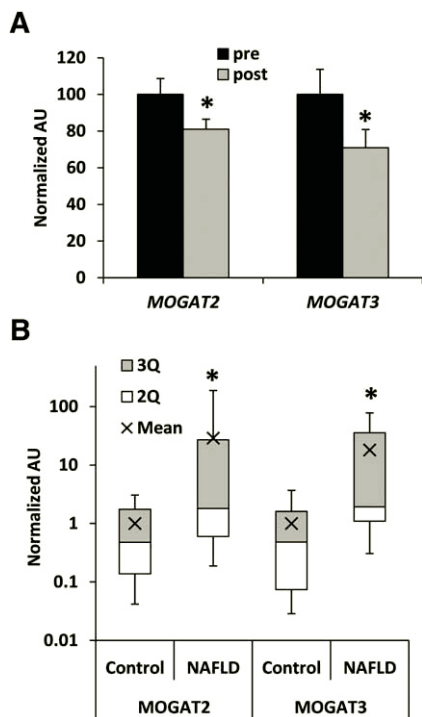


Fig. 7. *MOGAT2* and *MOGAT3* expression in obese subjects is diminished following marked weight loss and is induced in subjects with NAFLD. (A) Expression of *MOGAT2* and *MOGAT3* in liver of obese subjects at time of GBS and one year later. (B) Expression of *MOGAT2* and *MOGAT3* in liver of subjects with NAFLD and control subjects.

reports that the hepatic expression of *Mogat1* (22, 30) and *Lpgat1* (31) is upregulated in mouse models of hepatic steatosis and that the targeted knockdown of *Lpgat1* in liver of diet-induced obese mice reduced hepatic steatosis and insulin resistance (31). These studies conducted in mouse models are consistent with the present findings that *MOGAT2* and *MOGAT3* expression was robustly increased in liver of subjects with NAFLD and was significantly downregulated by marked weight loss in extremely obese individuals. It is difficult to determine the molecular or physiologic factors that influence the expression of *MOGAT2* and *MOGAT3* in these obese subjects. Gastric bypass surgery, in addition to causing marked weight loss, leads to a striking improvement in hepatic steatosis, insulin sensitivity, and other metabolic parameters (Table 1 and Refs. 32–34). Future studies will be needed to clarify the factors that regulate *MOGAT* expression in liver.

In summary, we found that the MGAT enzymes are active in human liver and that the expression of the genes encoding these enzymes is dynamically regulated. The present data are also consistent with the idea that MGAT enzyme activity could represent a viable target for pharmacologic intervention to treat hepatic insulin resistance and NAFLD.

The authors thank the study participants and research coordinators at Washington University School of Medicine. The authors also thank many employees of Pfizer Global Research and Development, including Adam Skepner for preparing the MGAT3 adenovirus, James Duerr for provision of

the expression construct, and George Tkalcovic and Timothy Subashi for recombinant human MGAT3 production.

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