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Abrogating Monoacylglycerol Acyltransferase Activity in Liver Improves Glucose Tolerance and Hepatic Insulin Signaling in Obese Mice

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Monoacylglycerol acyltransferase (MGAT) enzymes convert monoacylglycerol to diacylglycerol (DAG), a lipid that has been linked to the development of hepatic insulin resistance through activation of protein kinase C (PKC). The expression of genes that encode MGAT enzymes is induced in the livers of insulin-resistant human subjects with nonalcoholic fatty liver disease, but whether MGAT activation is causal of hepatic steatosis or insulin resistance is unknown. We show that the expression of *Mogat1*, which encodes MGAT1, and MGAT activity are also increased in diet-induced obese (DIO) and *ob/ob* mice. To probe the metabolic effects of MGAT1 in the livers of obese mice, we administered antisense oligonucleotides (ASOs) against *Mogat1* to DIO and *ob/ob* mice for 3 weeks. Knockdown of *Mogat1* in liver, which reduced hepatic MGAT activity, did not affect hepatic triacylglycerol content and unexpectedly increased total DAG content. *Mogat1* inhibition also increased both membrane and cytosolic compartment DAG levels. However, *Mogat1* ASO treatment significantly improved glucose tolerance and hepatic insulin signaling in obese mice. In summary, inactivation of hepatic MGAT activity, which is markedly increased in obese mice, improved glucose tolerance and hepatic insulin signaling independent of changes in body weight, intrahepatic DAG and TAG content, and PKC signaling.

Obesity is associated with intrahepatic lipid accumulation, which has been linked to the development of insulin resistance and metabolic dysfunction. For example, accumulation of diacylglycerol (DAG) is associated with insulin resistance, and experimentally modulating DAG levels affects hepatic insulin sensitivity (1–5). Similarly, other lipid mediators activate signaling cascades, leading to impairment in insulin sensitivity in liver (6). However, given the impossibility of modulating the concentration of one lipid species in isolation and the potential number of candidate lipids, the identity of lipids that link hepatic lipid accumulation and insulin resistance is still not completely understood. Furthermore, even the cause-and-effect relationship between hepatic steatosis and insulin resistance can be debated (7).

Triacylglycerol (TAG) is the primary storage form of intracellular lipids, and TAG is solely generated from acylation of DAG. In most cells of the body, DAG destined for TAG synthesis is produced primarily from the sequential acylation and dephosphorylation of glycerol-3-phosphate (Fig. 1*A*) (8,9). However, there are also alternative pathways for synthesizing DAG, including acylation of monoacylglycerol, which is catalyzed by monoacylglycerol acyltransferase (MGAT) enzymes (Fig. 1*A*). The human and mouse genomes each contain three MGAT family genes (*Mogat1, 2,* and *3*), but mouse *Mogat3* is a pseudogene and not analogous to the human MOGAT3 (10). The MGAT

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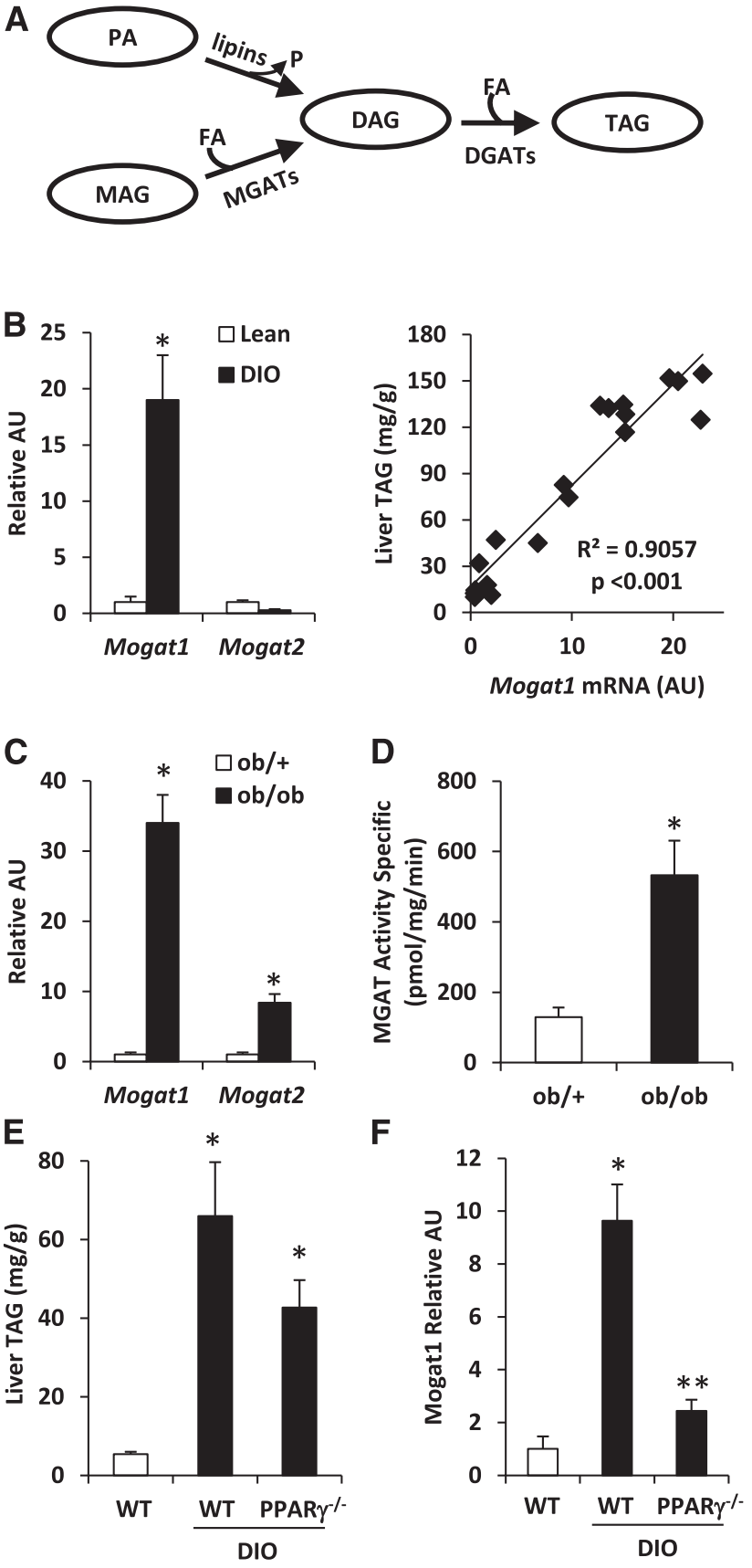

Figure 1—*Mogat1* expression is increased in obese mice in a PPARγ-dependent manner. *A*: The schematic depicts the two metabolic pathways for triglyceride synthesis. *B*: Hepatic *Mogat1* expression in mice fed chow containing 60% fat or 10% fat for 14 weeks. The scatter plot (*right*) depicts the relationship between liver TAG content and *Mogat* expression in individual mice. *C*: Hepatic *Mogat* expression in *ob*/*ob*

enzymes are important for dietary fat absorption by intestinal enterocytes, and *Mogat1* and *Mogat2* are most highly expressed in the gastrointestinal system (11–13). MGAT enzymes may also be an important mechanism for recycling remnants of lipolytic processes in nonintestinal cells (14,15), but relatively little is known about their effects in extraintestinal tissues.

MGAT enzymes are of potential relevance to mechanisms of obesity-related hepatic steatosis for a number of reasons. First, as noted, the product of MGAT activity (DAG) has been linked to the development of insulin resistance in a variety of tissues (3). Second, the expression of genes encoding MGAT enzymes has been shown to be induced in steatotic liver in mice (16) and human subjects (11). We have shown that marked weight loss in obese subjects after gastric bypass surgery leads to reduced expression of the MOGAT genes and that this coincides with insulin sensitization and resolution of hepatic steatosis (11). Finally, mice null for *Mogat2* are protected from diet-induced obesity due to delayed absorption of dietary fat and increased systemic energy expenditure (17).

To examine the metabolic consequences of reversing the activation of *Mogat1* in liver, antisense oligonucleotides (ASOs) targeting *Mogat1* for knockdown were administered to diet-induced obese (DIO) or *ob/ob* mice in which hepatic expression of *Mogat1* is markedly induced. To our surprise, attenuation of hepatic *Mogat1* expression led to increased DAG content in obese liver but significantly improved glucose tolerance and hepatic insulin signaling. These data suggest that targeting MGAT activity could be a novel strategy for improving obesity-related insulin resistance.

RESEARCH DESIGN AND METHODS

Animal Studies

All mice were maintained in accordance with the Animal Use and Care Committees of Washington University School of Medicine. To cause DIO, C57BL/6J male mice were fed chow providing 60% of calories from fatty acids (D12492; Research Diets, Inc.) starting at 6 weeks of age. Age-matched mice were maintained on a matched 10% fat chow (D12450B; Research Diets, Inc.). *Ob/ob* and *ob*/+ control mice were obtained at 6 weeks of age from The Jackson Laboratory (Bar Harbor, ME). Mice with liver-specific peroxisome proliferator–activated receptor (PPAR) γ deficiency have been described (18) and were compared with littermate $PPAR\gamma^{flox/flox}$ mice not expressing Cre.

Mice received intraperitoneal injections of ASO directed against *Mogat1* or a scrambled control ASO 25 mg/kg body weight (ISIS Pharmaceuticals, Inc., Carlsbad, CA) twice a week for 3 weeks. Treatments were initiated after 14 weeks of high-fat-diet feeding or at 6 weeks of age in *ob/ob* mice. After treatment with ASOs for 18 days, mice were subjected to a glucose tolerance test. Mice were killed after 3 weeks of injections with ASOs, and tissues were harvested, frozen in liquid nitrogen, and stored at −80°C for further analyses.

Glucose tolerance tests were performed as described (19). Before the tests, mice were fasted for 4 h and then injected with a 10% solution of D-glucose (1 g/kg). Tail blood glucose was determined at 0, 30, 60, and 120 min after challenge using a OneTouch Ultra glucometer (LifeScan, Inc.). Total area under the curve was calculated using the trapezoidal rule. In one study, plasma was collected by mandibular bleed at the 15-min time point for assessment of insulin concentration.

Insulin Signaling

To assess insulin signaling in peripheral tissues, mice were fasted for 4 h before being killed. At time 0, mice received an intraperitoneal injection of 10 mU/g body weight insulin or an equal volume of saline. Five minutes after the injection, tissues (liver, gastrocnemius, and white adipose tissue [WAT]) were removed, immediately frozen in liquid nitrogen, and stored at −80°C for further analyses.

Plasma Metabolite Quantification

Nonesterified fatty acids were measured using commercially available kits (NEFA-HR; Wako Diagnostics, Richmond, VA). TAG and cholesterol content were determined using colorimetric kits from Thermo Scientific as previously described (20). Plasma insulin concentrations were determined by a commercially available ELISA (Crystal Chemical Company, Wakefield, MA).

MGAT and DAG Acyltransferase Enzymatic Assays

Membrane fractions were isolated from mouse liver by homogenization in 50 mmol/L Tris-HCl (pH 7.4), 1 mmol/L EDTA, and 0.25 mol/L sucrose and centrifuging at 100,000*g* for 1 h. Fifty micrograms of protein were incubated in 5 mmol/L $MgCl_2$, 1.25 mg/mL BSA, 200 mmol/L sucrose, 100 mmol/L Tris-HCl (pH 7.4), 25 μmol/L [^{14}C]oleoyl-CoA, and 200 μmol/L sn-2-oleoylglycerol (for MGAT assay) or 200 μmol/L sn-1,2-dioleoylglycerol (for DAG acyltransferase [DGAT] assay) for 5 min. The reaction was terminated by the addition of 50 μL 1% phosphoric acid. Lipids were extracted with 300 μL $CHCl_3$/MeOH (2:1 volume for volume) and separated by thin-layer chromatography (TLC) with hexane/ethyl ether/acetic acid (80:20:1 volume for volume for volume). Lipids were extracted and separated on Linear-K preadsorbent TLC plates. The spots corresponding to DAG and TAG were scraped from the TLC plates and ^{14}C-radioactivity was quantified by a scintillation counter.

and lean *ob*/+ control mice. *D*: Hepatic MGAT activity in *ob/ob* and lean *ob*/+ control mice. *E*: Hepatic TAG content in WT or liver-specific $PPAR\gamma^{-/-}$ mice fed a low- or high-fat diet for 14 weeks. *F*: Hepatic *Mogat1* expression in WT or liver-specific $PPAR\gamma^{-/-}$ mice fed a low- or high-fat diet for 14 weeks. **P* < 0.05 vs. lean controls; ***P* < 0.05 vs. lean and DIO WT mice. AU, arbitrary unit; FA, fatty acid; MAG, monoacylglycerol; P, phosphate; PA, phosphatidic acid.

Reaction mixtures in which liver membrane proteins were omitted were used as blanks for background correction.

mRNA Isolation and Gene Expression Analyses

Total liver RNA was extracted with RNA-BEE (Iso-Tex Diagnostics, Friendswood, TX) according to the manufacturer's instructions. First-strand cDNA synthesis was performed using Taqman reagents, and quantitative real-time RT-PCR was performed using the ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA) with Power SYBR green. Arbitrary units of target mRNA were corrected to the corresponding level of the 36B4 mRNA. The sequences of the primers used in these studies are available upon request.

Western Blot Analyses

For Western blot analyses using whole-cell lysates, protein extracts were obtained from liver by using the following lysis buffer containing a protease inhibitor cocktail: 10 mmol/L HEPES (pH 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EDTA, 0.1% sodium deoxycholate, and 1% Triton X-100. For subcellular protein kinase C (PKC) localization studies, livers from lean, DIO, and DIO ASO–treated mice were fractionated as previously described (21). Briefly, 100 mg of tissue was homogenized by a Potter-Elvehjem homogenizer in lysis buffer (10 mmol/L HEPES, 1 mmol/L EDTA [pH 7.4]) with 60% sucrose. The homogenates were transferred to 2-mL centrifuge tubes. The tubes were then filled to capacity with lysis buffer. The gradients were centrifuged at 20,000*g* for 3 h at 4°C. Fractions were collected as described previously and stored at −80°C until Western blot analyses (21). Antibodies to Akt, pSer473 Akt, GSKα/β, pSer21/9 GSKα/β (Cell Signaling Technology, Danvers, MA), actin (Sigma-Aldrich, St. Louis, MO), PKCε (BD Biosciences, San Jose, CA), PKCδ (Santa Cruz Biotechnology, Dallas, TX), and calnexin (Enzo Life Sciences, Farmingdale, NY) were used according to the manufacturers' instructions.

PKC Enzymatic Assay

Total membrane-associated PKC activity was quantified using the Protein Kinase C Biotrak Enzymatic Assay System (GE Healthcare, Chalfont St. Giles, Buckinghamshire, U.K.) following the manufacturer's protocol. The assay was initiated by the addition of 1 μg of membrane proteins to the reaction component mixture and incubated at 37°C for 15 min. Reactions were terminated with the addition of 300 mmol/L orthophosphoric acid and processed as described by the manufacturer. Protein lysates from mouse brain, which contain high PKC activity, were used as positive control.

Tissue Glycerolipid Analyses

Total hepatic TAG content was determined using a colorimetric assay as previously described (20). Hepatic DAG content was separated into membrane and cytoplasmic fractions as previously described (22). Briefly, samples were homogenized on ice using a dounce homogenizer in 500 μL of lysis buffer containing 10 mmol/L Tris base, 0.5 mmol/L EDTA, 250 mmol/L sucrose, and protease inhibitors. Samples were centrifuged at 100,000*g* for 1 h. The supernatant and lipid layers were removed and designated as the cytoplasmic fraction. The pellet was resuspended in lysis buffer and designated as the membrane fraction.

Concentrations of DAG molecular species in each fraction were determined by using mass spectrometry as previously described (23). Neutral lipids were extracted by hexane, and the free hydroxyl group in DAG species was protected with 2,4-difluorophenyl isocyanate. The analysis was performed on an Agilent 1100 LC system connected to an Agilent 6460 TSQ mass spectrometer operated in electrospray ionization and positive ion mode (24). The liquid chromatography system allows separation of DAG species from other neutral lipids and resolution of sn-1,2 DAG and sn-1,3 DAG species.

Statistical Analyses

All data are presented as the mean ± SE. Statistical significance was calculated using an unpaired Student *t* test, with a statistically significant difference defined as $P \leq 0.05$.

RESULTS

Expression of *Mogat1* Is Induced in Liver of Obese Mice

We examined *Mogat1* expression in the livers of mice made obese by 14 weeks of high-fat-diet feeding. As expected, mice fed a high-fat diet were obese and had elevated liver TAG content compared with low-fat-diet–fed control mice (data not shown). The hepatic expression of *Mogat1* was strongly induced in DIO mice compared with lean controls, whereas the expression of *Mogat2* was not markedly affected (Fig. 1*B*). The expression of *Mogat1* was tightly correlated (R^2 = 0.9057) with hepatic TAG content in the livers of lean and DIO mice (Fig. 1*B*). Hepatic *Mogat1* expression and MGAT activity were also increased in *ob/ob* mice compared with lean controls (Fig. 1*C* and *D*). *Mogat2* was induced in *ob/ob* liver compared with that in lean controls (Fig. 1*C*). Collectively, these data indicate that hepatic expression of *Mogat1* and MGAT activity are increased in livers of mice that are obese due to dietary intervention or genetic defects.

Induction of *Mogat1* Expression by DIO Requires PPARγ

Mogat1 has been shown to be a direct target gene for PPARγ (16). To evaluate the requirement for PPARγ in regulating *Mogat1* expression in liver of high-fat-diet–fed mice, we generated mice with liver-specific PPARγ deletion (LS-PPARγ$^{-/-}$ mice) (18) and induced them to become obese by feeding with a high-fat diet for 16–20 weeks. Although LS-PPARγ$^{-/-}$ mice tended to accumulate less hepatic TAG than wild-type (WT) DIO mice, hepatic TAG levels remained elevated in DIO LS-PPARγ$^{-/-}$ mice compared with lean controls (Fig. 1*E*). The induction of *Mogat1* in response to DIO was markedly attenuated in LS-PPARγ$^{-/-}$ mice compared with WT DIO mice (Fig. 1*F*). These data suggest that the induction of *Mogat1* in the livers of DIO mice requires PPARγ and are consistent

with previous work demonstrating that *Mogat1* is a direct target of PPARγ.

Mogat1 Knockdown Abolishes the Increase in Hepatic MGAT Activity in Liver of Obese Mice

We evaluated the consequences of knocking down *Mogat1* expression using ASO technology. Administration of ASO targeting *Mogat1* to DIO mice that had been fed a high-fat diet for 14 weeks did not affect body weight gain or epididymal fat pad weight (Fig. 2*A*) but did result in a marked reduction in hepatic *Mogat1* expression (DIO ASO) compared with DIO mice injected with scrambled control ASO (DIO) (Fig. 2*B*). *Mogat1* ASO did not affect the expression of *Mogat2* (Fig. 2*B*), and knockdown of *Mogat1* was restricted to liver because the expression of *Mogat1* was not affected in stomach, skeletal muscle (SKM), or WAT (data not shown). *Mogat1* ASO administration resulted in a reduction in hepatic MGAT activity (Fig. 2*B*). ASO treatment also reduced expression of *Mogat1* and *Mogat2* as well as MGAT activity in the livers of *ob/ob* mice (Fig. 2*C*).

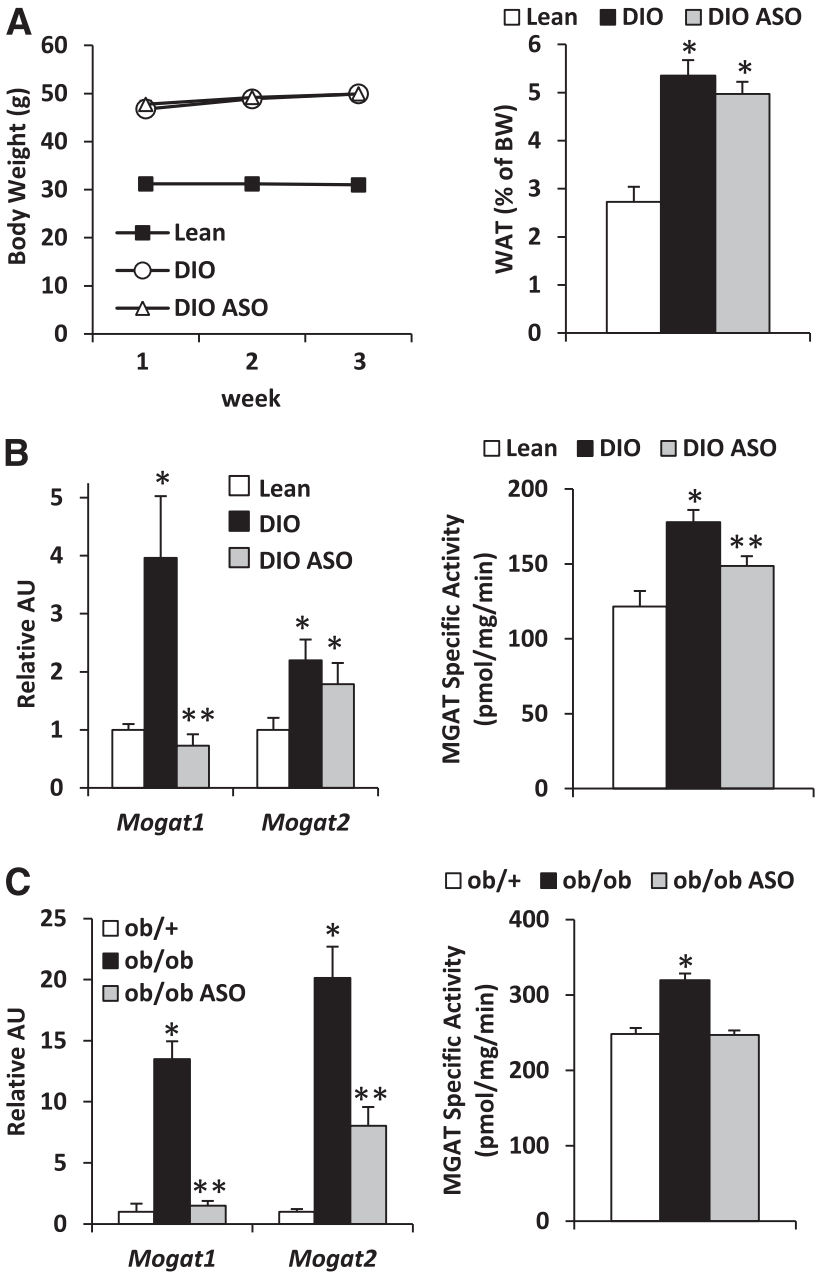

Figure 2—*Mogat1* ASO reduces hepatic MGAT activity in DIO and *ob/ob* mice. *A*: Body weight and epididymal fat pad weight of mice fed chow containing 60% fat or 10% fat for 14 weeks and treated with ASO against *Mogat1* or scrambled control ASO for 3 weeks. *B*: *Mogat* expression and MGAT activity in liver of mice treated with ASO against *Mogat1* or scrambled control ASO. **P* < 0.05 vs. lean controls; ***P* < 0.05 vs. lean and DIO control ASO mice. *C*: Hepatic *Mogat* expression and MGAT activity of *ob/ob* mice treated with ASO against *Mogat1* or scrambled control ASO. **P* < 0.05 vs. lean controls; ***P* < 0.05 vs. lean and *ob/ob* control ASO mice. AU, arbitrary unit; BW, body weight.

Diminished *Mogat1* Expression in Obese Liver Does Not Affect Hepatic TAG Content and Increases DAG Levels

Hepatic TAG content in DIO and *ob/ob* mice was markedly increased compared with lean controls, and treatment with *Mogat1* ASO did not affect TAG levels (Fig. 3*A*). Hepatic ceramide content was also not affected by *Mogat1* ASO (data not shown). Mass spectrometry analyses demonstrated that hepatic DAG content, which was increased in the livers of DIO and *ob/ob* mice compared with lean controls, was unexpectedly increased further by *Mogat1* knockdown in both types of obese mice (Fig. 3*B*). These data suggest that knockdown of *Mogat1* does not reduce but actually increases hepatic DAG content. One possible explanation for this observation is that hepatic DGAT activity was also decreased by *Mogat1* ASO coincident with reduced expression of *Dgat2* in the livers of mice treated with *Mogat1* ASO (Fig. 3*C*).

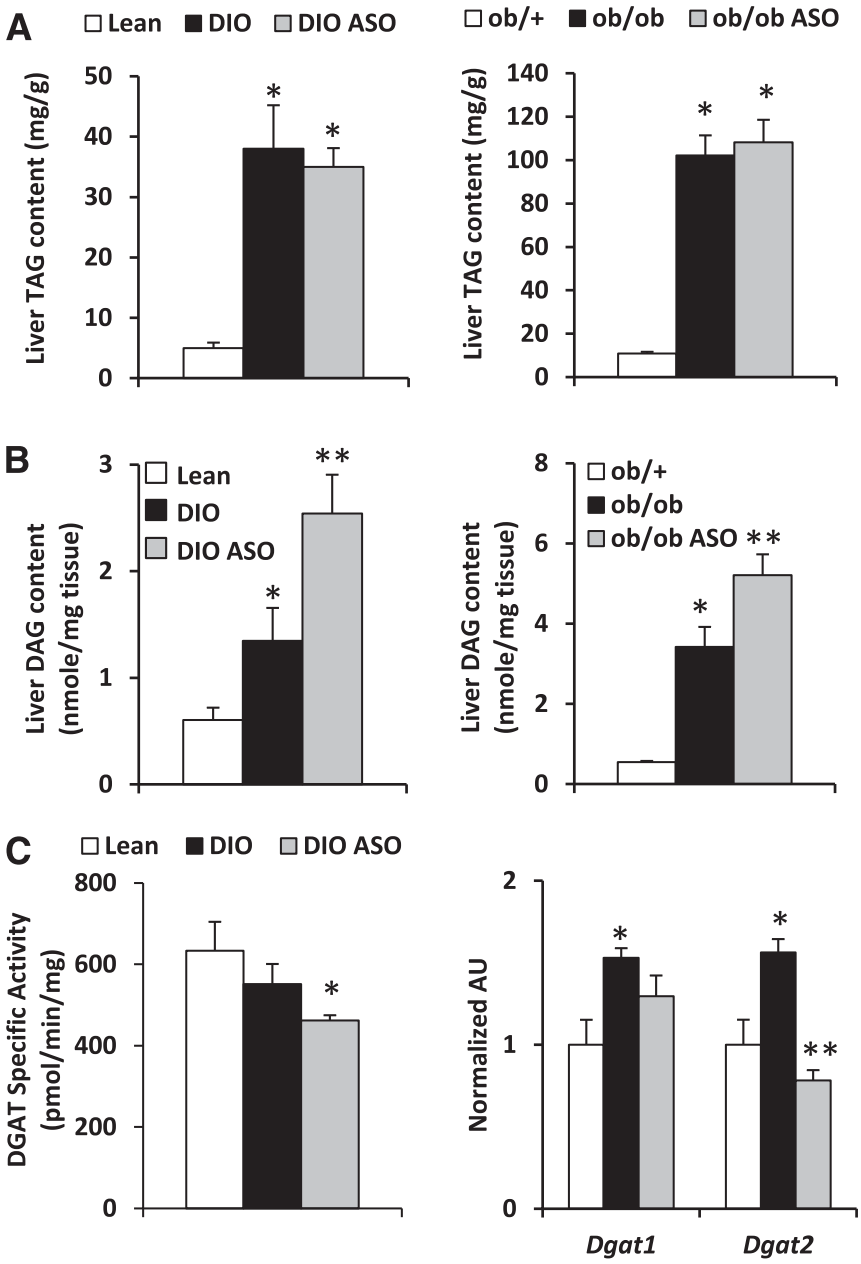

Figure 3—*Mogat1* knockdown does not affect liver TAG content but increases DAG levels. *A*: Hepatic TAG content in mice fed chow containing 60% fat or 10% fat for 14 weeks (*left*) or *ob/ob* mice (*right*) and treated with ASO against *Mogat1* or scrambled control ASO. *B*: Hepatic DAG content in mice fed chow containing 60% fat or 10% fat for 14 weeks (*left*) or *ob/ob* mice (*right*) and treated with ASO against *Mogat1* or scrambled control ASO. *C*: Hepatic DGAT activity and *Dgat* expression of mice fed chow containing 60% fat or 10% fat for 14 weeks and treated with ASO against *Mogat1* or scrambled control ASO. **P* < 0.05 vs. lean controls; ***P* < 0.05 vs. lean and obese control ASO mice. AU, arbitrary unit.

Inhibition of *Mogat1* Expression Reduces the Expression of Several Known Genes Activated in Steatotic Liver

We next evaluated the effects of *Mogat1* knockdown on the hepatic expression of a number of lipogenic genes known to be activated in insulin-resistant liver. Of note, *Mogat1* knockdown reduced the expression of PPARγ (*Pparg*) and its known target genes *Cidec*, *Cfd, Vldlr,* and *Plin4* but not *Cd36* and *Fabp4* (Fig. 4). The expression of sterol regulatory element–binding protein 1 (SREBP1 [*Srebf1*]) and its target genes *Fasn* and *Cidea*, which were increased in DIO mice compared with lean control mice, was also corrected by *Mogat1* ASO treatment. These data are consistent with reduced expression and activity of the lipogenic transcription factors PPARγ and SREBP1 in DIO liver after treatment with *Mogat1* ASO.

Inhibition of *Mogat1* Expression Improves Insulin Sensitivity

We next examined the effects of *Mogat1* knockdown on measures of circulating metabolite levels, glucose metabolism, and insulin sensitivity. *Mogat1* ASO treatment did not significantly affect plasma TAG, free fatty acid, or cholesterol concentration in DIO mice (Table 1). *Ob/ob*

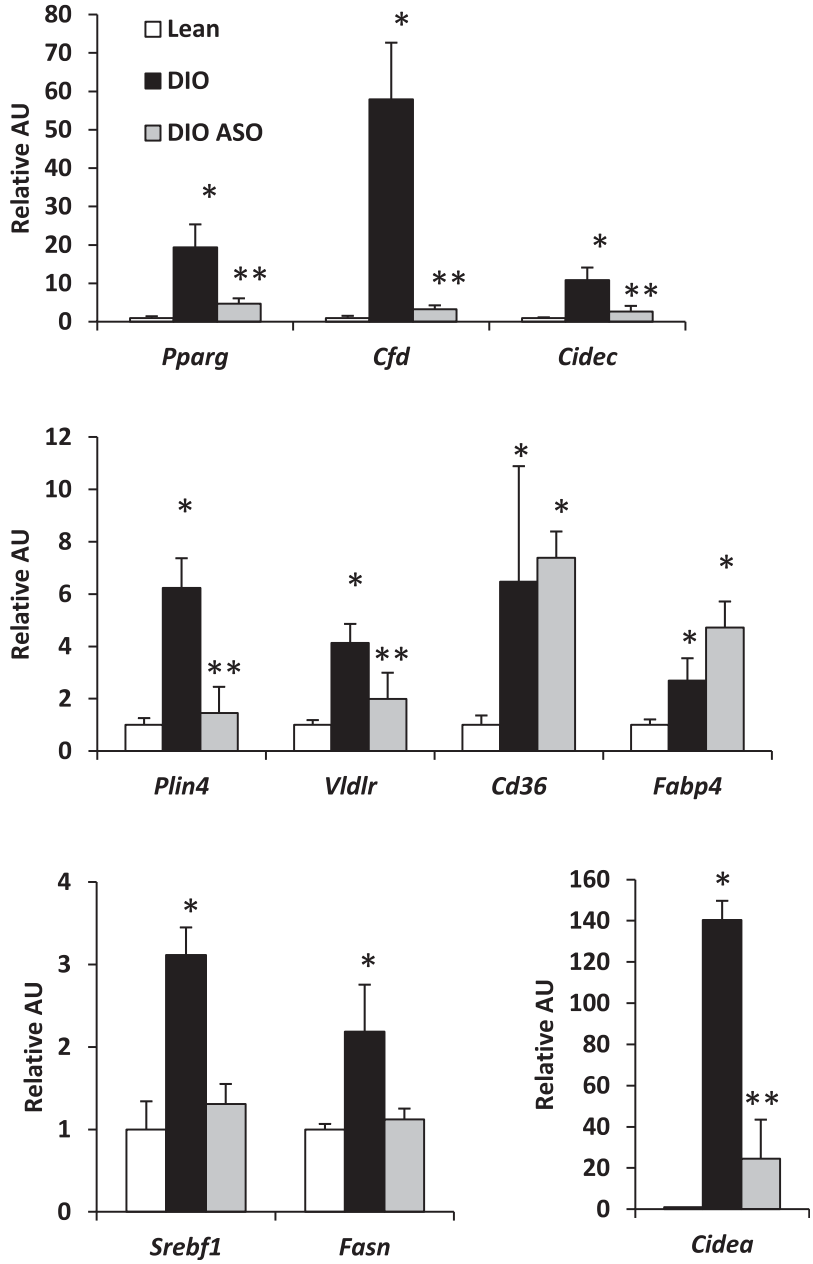

Figure 4—*Mogat1* ASO reduces hepatic expression of lipogenic genes in DIO mice. Hepatic expression of the indicated genes in mice fed chow containing 60% fat or 10% fat for 14 weeks and treated with ASO against *Mogat1* or scrambled control ASO. *$P < 0.05$ vs. lean controls; **$P < 0.05$ vs. lean and DIO control mice. AU, arbitrary unit.

Table 1—Plasma parameters

	Lean	DIO control ASO	DIO *Mogat1* ASO
TAG (mg/dL)	60.4 ± 4.0	53.8 ± 2.5	57.1 ± 4.1
NEFA (mmol/L)	0.57 ± 0.07	0.51 ± 0.04	0.51 ± 0.03
Cholesterol (mg/dL)	48.8 ± 2.5	101.1 ± 13.5*	107.2 ± 12.4*
Insulin (ng/mL)	1.6 ± 0.2	4.5 ± 1.1*	1.9 ± 0.4**
	ob/+	*ob/ob* control ASO	*ob/ob Mogat1* ASO
TAG (mg/dL)	55.5 ± 17.6	130.1 ± 19.2*	71.9 ± 7.3**
NEFA (mmol/L)	0.9 ± 0.1	1.8 ± 0.2*	1.6 ± 0.1*
Cholesterol (mg/dL)	99.5 ± 12.8	168.9 ± 18.0*	190.2 ± 24.4*
Insulin (ng/mL)	0.9 ± 0.1	9.9 ± 0.8*	7.6 ± 1.0**

Data are mean ± SE. Plasma was collected at sacrifice after a 4-h fast. NEFA, nonesterified fatty acid. *$P < 0.05$ vs. lean controls. **$P < 0.05$ vs. ASO control and lean controls.

mice, which were hypertriglyceridemic, exhibited a marked decrease in plasma TAG concentration in response to *Mogat1* knockdown, whereas plasma cholesterol and free fatty acid concentrations were not affected. Plasma insulin concentration was reduced in DIO and *ob/ob* mice by treatment with *Mogat1* ASO compared with control ASO-treated mice (Table 1), suggesting that sensitivity to this hormone might be increased. Glucose tolerance testing demonstrated that *Mogat1* ASO treatment markedly improved glucose tolerance compared with control ASO treatment in DIO mice (Fig. 5*A*). The improvement in glucose tolerance was not associated with increased insulin secretion because the plasma insulin concentration 15 min after glucose injection was actually reduced in *Mogat1* ASO-treated mice compared with DIO controls and the insulin-to-glucose ratio was unchanged (Fig. 5*A*). Glucose tolerance was also improved in *ob/ob* mice treated with *Mogat1* ASO compared with control ASO-treated mice (Fig. 5*B*).

To examine hepatic insulin signaling, we evaluated the phosphorylation of proteins downstream of the insulin receptor in liver after maximal insulin stimulation. The insulin-stimulated phosphorylation of Akt at Ser473, Akt at Thr308, and GSK3α at Ser21 was increased in the livers of DIO mice treated with *Mogat1* ASO compared with mice injected with control ASO (Fig. 5*C*). In contrast, insulin-stimulated phosphorylation of Akt at Ser473 in SKM and WAT was not affected by *Mogat1* knockdown (Fig. 5*D*). These data indicate that *Mogat1* inhibition in liver improves systemic glucose tolerance and hepatic insulin signaling.

Mogat1 ASO Increases the Ratio of Membrane-to-Cytosolic–Associated PKCs

The data presented previously suggest that *Mogat1* knockdown improves glucose tolerance and hepatic insulin signaling despite increases in total cellular DAG content. Previous studies also detecting increased DAG in insulin-sensitized mice reconciled these findings by identifying correlations between DAG in membrane or cytoplasmic compartments in the cell and insulin resistance (22). However, we found that both membrane and cytosolic DAG content was increased by *Mogat1* knockdown (Fig. 6*A*). In addition, DAG with saturated fatty acids esterified at the sn-1,2 position are believed to be more robust activators of PKC signaling than sn-1,3 DAG species (25–27). We found that *Mogat1* inhibition led to an accumulation of total sn-1,2 DAG (Fig. 6*B*), and all sn-1,2 DAG species tended to be or were significantly increased in DIO versus lean liver and further increased by *Mogat1* ASO treatment, including a number of saturated fatty acid species (Supplementary Fig. 1). The only remarkable change in DAG species was that sn-1,3 DAG was specifically increased in DIO mice treated with *Mogat1* ASO, and the ratio of sn-1,3 to sn-1,2 DAG, which is reduced in DIO compared with lean mice, was increased by *Mogat1* knockdown (Fig. 6*B*).

Other work has suggested that activation of PKCε and PKCδ by DAG is involved in the development of hepatic insulin resistance (1,28–32). We found that the expression of mRNA encoding PKCε (Prcke) but not PKCδ (Prckd) was increased by DIO and significantly reduced by treatment with *Mogat1* ASO (Fig. 6*C*). However, the total cellular protein abundance of PKCε and PKCδ was not affected by DIO and was diminished after *Mogat1* ASO treatment (Fig. 6*D*). The activation of PKCs often is assessed by quantifying the membrane association of PKC protein (1,28–30). Although we found no effect of DIO on PKCε or PKCδ membrane association or membrane-associated PKC activity, *Mogat1* knockdown significantly decreased membrane-associated PKC activity in DIO mice (Fig. 6*F*), which tracks with the abundance of membrane-associated PKCε (Fig. 6*C*). Collectively, the data suggest that knocking down *Mogat1* in liver of obese mice improves glucose metabolism and insulin signaling independent of reductions in intrahepatic DAG or TAG content.

DISCUSSION

The mechanisms whereby caloric excess and obesity lead to hepatic steatosis continue to emerge. Work from our group and others has shown that obese mice and human subjects exhibit a marked activation in the expression of

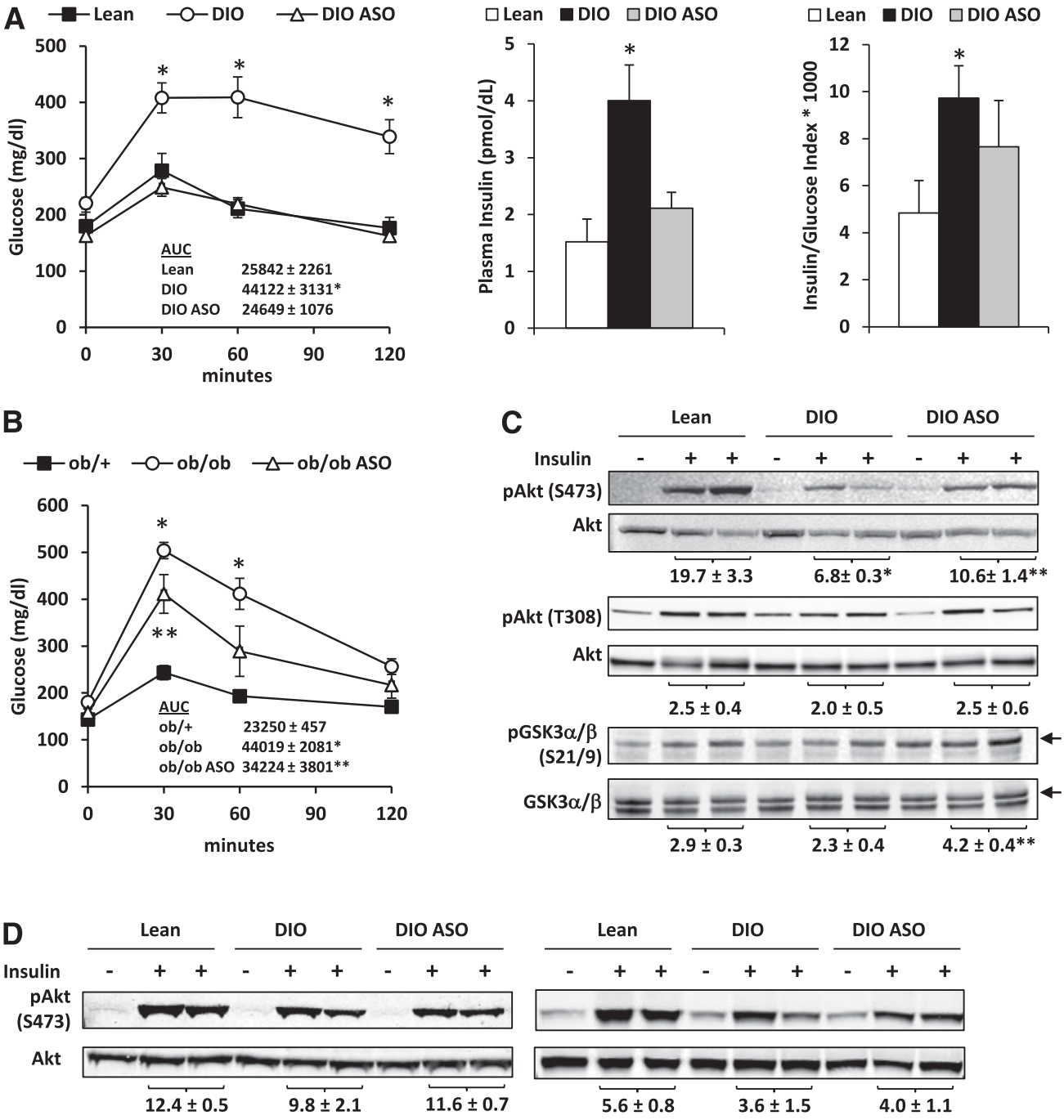

Figure 5—Inhibition of *Mogat1* improves systemic glucose tolerance and hepatic insulin signaling. *A*: Glucose tolerance tests (GTTs) in DIO mice treated with ASO against *Mogat1* or scrambled control ASO. AUC values are inset. Plasma insulin concentration (*middle panel*) and insulin/glucose index (*right panel*) were determined at the 15-min time point during the GTT. **P* < 0.05 vs. lean controls. *B*: GTTs in *ob/ob* mice treated with ASO against *Mogat1* or scrambled control ASO. AUC values are inset. **P* < 0.05 vs. lean controls; ***P* < 0.05 vs. lean and *ob/ob* control ASO mice. *C*: Representative Western blots for liver lysates and the indicated antibodies. Five minutes before being killed, mice were injected with saline or insulin. Quantification of insulin-stimulated phosphorylation (relative to the total protein) is inset below each blot. Arrows indicate the bands for GSK3α. **P* < 0.05 vs. lean controls; ***P* < 0.05 vs. DIO control ASO mice. *D*: Representative blots of gastrocnemius SKM and epididymal WAT from mice fed chow containing 60% fat or 10% fat for 14 weeks and treated with ASO against *Mogat1* or scrambled control ASO. AUC, area under the curve.

the genes encoding MGAT enzymes, which catalyze the penultimate step in TAG synthesis (11,16). In this work, we found that *Mogat1*, which is expressed at low levels in normal mouse liver, is robustly induced by obesity through activation of PPARγ. Knockdown of *Mogat1* in liver of obese mice improves circulating TAG concentration, glucose tolerance, and hepatic insulin signaling without reducing body weight or intrahepatic DAG or TAG

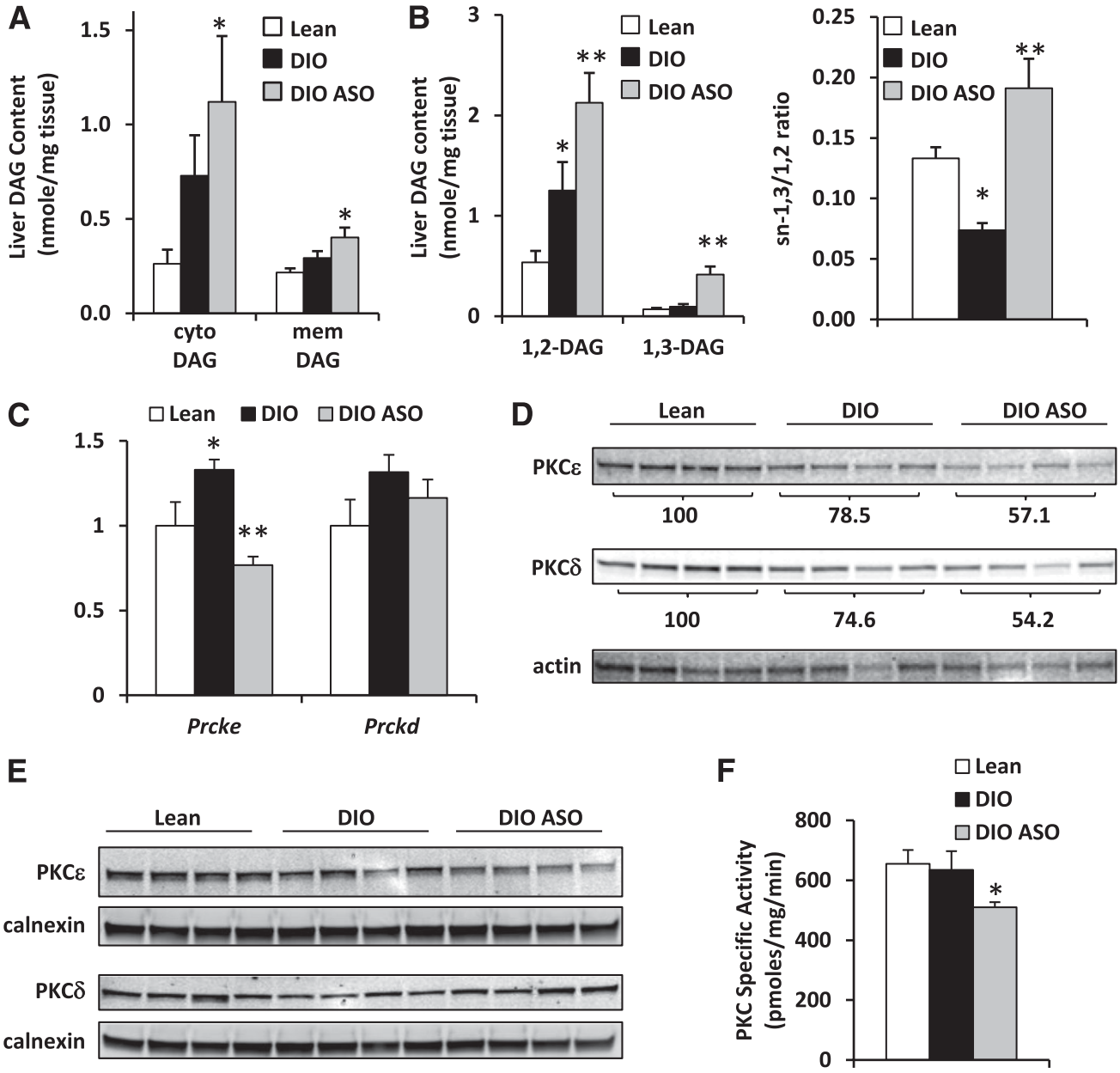

Figure 6—*Mogat1* knockdown increases membrane DAG content and association with PKC isoforms. *A*: Cytoplasmic and membrane DAG content in livers of mice fed chow containing 60% fat or 10% fat for 14 weeks (*left*) and treated with ASO against *Mogat1* or scrambled control ASO. **P* < 0.05 vs. lean mice. *B*: Hepatic sn-1,2 and sn-1,3 DAG content in mice fed chow containing 60% fat or 10% fat for 14 weeks (*left*) and treated with ASO against *Mogat1* or scrambled control ASO. The ratio of sn-1,3 to sn-1,2 DAG was also calculated (*right*). **P* < 0.05 vs. lean controls; ***P* < 0.05 vs. lean and obese control ASO mice. *C*: Hepatic expression of Prcke and Prckd from liver of mice fed chow containing 60% fat or 10% fat for 14 weeks and treated with ASO against *Mogat1* or scrambled control ASO for 3 weeks. **P* < 0.05 vs. lean controls; ***P* < 0.05 vs. lean and obese control ASO mice. *D*: Representative Western blots for total cellular PKCε and PKCδ from livers of mice fed chow containing 60% fat or 10% fat for 14 weeks and treated with ASO against *Mogat1* or scrambled control ASO for 3 weeks. Quantification of PKC isoform expression (relative to actin expression) is inset below each blot. *E*: Representative Western blots for PKCε and PKCδ in membrane protein fractions from livers of mice fed chow containing 60% fat or 10% fat for 14 weeks and treated with ASO against *Mogat1* or scrambled control ASO for 3 weeks. *F*: Membrane-associated PKC-specific activity from liver of mice fed chow containing 60% fat or 10% fat for 14 weeks and treated with ASO against *Mogat1* or scrambled control ASO for 3 weeks. **P* < 0.05 vs. lean and obese control ASO mice. Cyto, cytoplasmic; mem, membrane.

content. These data suggest that targeting hepatic MGAT activity, which is also increased in the livers of obese humans, might have promise in treating dyslipidemia, insulin resistance, and diabetes.

Intrahepatic lipid content is well correlated with systemic insulin sensitivity and the circulating concentrations of cardiometabolic risk factors (33). Although the cause-and-effect relationship between hepatic steatosis

and insulin sensitivity is unclear, there is evidence that several lipid intermediates in the TAG synthesis pathway may activate signaling pathways that lead to impaired insulin signaling (34). For example, DAG has been linked to insulin resistance through activation of novel-type PKCs (25). Membrane-associated DAG is known to activate PKCs (25–27), and the PKCε isoform has been shown to translocate from the cytosol to the membrane fraction, which suggests activation, in response to high-fat-diet feeding in rats (35). PKCε is believed to act at the membrane to impair insulin receptor kinase activity to blunt downstream effects on insulin signaling (35). A relationship between PKCε membrane localization and insulin resistance has been detected in a number of models (1,29,36,37). In support of this, administration of ASOs to knock down PKCε in rats fed a high-fat diet for 3 days improved hepatic insulin sensitivity (30), and PKCε knockout mice showed improved glucose tolerance while on a high-fat diet (32). However, the increased glucose tolerance in PKCε knockout mice seems to be due to exaggerated insulin secretion, suggesting a β-cell effect (32). Similarly, PKCδ is activated in liver of some obesity models, and PKCδ null mice are insulin sensitive while on a high-fat diet (31). In the current study, we found no increase in PKCε or PKCδ protein abundance or membrane localization or membrane-associated PKC activity after 17 weeks of feeding a 60% fat diet to mice. However, treatment with *Mogat1* ASO reduced the total cellular PKCε and PKCδ abundance and membrane-associated PKC activity, which is surprising given the link between DAG and PKC signaling and the observed increase in membrane DAG content. These findings suggest that the effects of *Mogat1* knockdown on the hepatic insulin signaling cascade are actually associated with increased hepatic DAG content.

In the current study, *Mogat1* knockdown indeed led to increased total hepatic DAG content. The molecular explanation for this is unclear because we did not see a compensatory increase in phosphatidic acid phosphohydrolase or TAG hydrolase activity (data not shown). We did observe a decrease in the activity of DGAT, which could account for DAG accumulation. A counterintuitive accumulation of DAG was also observed with DGAT overexpression in muscle and liver (38,39) and with knockdown of adipose tissue triglyceride lipase (40); thus, the regulation of the intracellular concentration of this lipid remains enigmatic. Other studies have also detected normal or increased insulin sensitivity in the context of increased DAG (4,39–43). Some work suggested that sequestering DAG in cytosolic lipid droplets, possibly in an innocuous storage depot, prevents accumulation of DAG in the membrane where it could attract PKCε translocation and lead to impaired insulin signaling (22). This contradicts previous work that found a positive correlation between cytosolic DAG content and insulin resistance and PKCε activation (44). Nonetheless, in the current study, *Mogat1* knockdown led to insulin sensitization despite causing increases in both membrane and cytosolic DAG content, suggesting that DAG sequestration does not explain the observed phenotype. We also found increases in all species of DAG after ASO treatment, including 16:0/18:1, 16:1/18:1, and other saturated fatty acid–containing DAG, which are best correlated with insulin resistance in SKM (45). Because most lipids are intermediates in metabolic pathways and are rapidly interconverted, it is nearly impossible to affect the concentration of one lipid in isolation, suggesting that changes in other lipid species mediate the effects of *Mogat1* knockdown. Future work will be needed to identify the species involved.

While the present article was in preparation, Lee et al. (16) reported that *Mogat1* expression is activated by PPARγ and that adenovirus-mediated knockdown of *Mogat1* improved insulin sensitivity in obese mice. In their work, hepatic *Mogat1* knockdown with adenoviral-driven short hairpin RNA led to dramatic body weight loss and attenuated hepatic steatosis, whereas we note no effect on these parameters after 3 weeks of *Mogat1* ASO treatment. It is likely that the observed reduction in intrahepatic TAG content in the work of Lee et al. is secondary to weight loss because this can rapidly reduce hepatic steatosis (46), and overexpression of *Mogat1* in liver is not sufficient to drive increased TAG content (A.M.H., unpublished observations). Indeed, the primary pathway for TAG synthesis in liver is through the sequential acylation of glycerol-3-phosphate, and this pathway does not require MGAT activity to synthesize DAG or TAG. It is still unclear why knockdown of *Mogat1* in liver in the Lee et al. study led to body weight loss because food intake and energy expenditure were not examined. It remains to be determined whether this was a nonspecific effect of the adenovirus infection.

In summary, inactivation of hepatic MGAT activity, which is markedly increased in obese mice, improved glucose tolerance and hepatic insulin signaling independent of changes in body weight, intrahepatic DAG and TAG content, or PKC signaling. Although it should be noted that the human ortholog of MGAT1 is poorly expressed and that MGAT3 may be the MGAT family member most highly expressed in human liver (11), targeting this enzymatic activity by pharmacologic means might be a viable therapeutic target for the treatment of diabetes. Additional work is needed to examine the mechanisms of insulin sensitization and to determine whether this approach is efficacious.

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