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Obesity Reduces Maternal Blood Triglyceride Concentrations by Reducing Angiopoietin-Like Protein 4 Expression in Mice

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To ensure fetal lipid supply, maternal blood triglyceride (TG) concentrations are robustly elevated during pregnancy. Interestingly, a lower increase in maternal blood TG concentrations has been observed in some obese mothers. We have shown that high-fat (HF) feeding during pregnancy significantly reduces maternal blood TG levels. Therefore, we performed this study to investigate if and how obesity alters maternal blood TG levels. Maternal obesity was established by prepregnant HF (ppHF) feeding, which avoided the dietary effect during pregnancy. We found not only that maternal blood TG concentrations in ppHF dams were remarkably lower than in control dams but also that the TG peak occurred earlier during gestation. Hepatic TG production and intestinal TG absorption were unchanged in ppHF dams, but systemic lipoprotein lipase (LPL) activity was increased, suggesting that increased blood TG clearance contributes to the decreased blood TG concentrations in ppHF dams. Although significantly higher levels of UCP1 protein were observed in interscapular brown adipose tissue (iBAT) of ppHF dams, *Ucp1* gene deletion did not restore blood TG concentrations in ppHF dams. Expression of the angiopoietin-like protein 4 (ANGPTL4), a potent endogenous LPL inhibitor, was significantly increased during pregnancy. However, the pregnancy-induced elevation of blood TG was almost abolished in *Angptl4*$^{-/-}$ dams. Compared with control dams, *Angptl4* mRNA levels were significantly lower in iBAT, gonadal white adipose tissue, and livers of ppHF dams. Importantly, ectopic overexpression of ANGPTL4 restored maternal blood TG concentrations in ppHF dams. Together, these results indicate that ANGPTL4 plays a vital role in increasing maternal blood TG concentrations during pregnancy. Obesity impairs the rise of maternal blood TG concentrations by reducing ANGPTL4 expression in mice.

Due to an increase in hepatic triglyceride (TG) secretion and reduction in plasma TG clearance, maternal blood TG concentrations progressively increase during pregnancy (1–3). Maternal blood TGs provide lipids not only for fetal growth but also as energy substrates for placental metabolism. Maternal blood TG levels are positively associated with birth weight in both healthy pregnancies (1,4,5) and pregnancies complicated with obesity and gestational diabetes (5–9). After adjustment for maternal BMI and blood glucose, maternal blood TG concentration has been identified as a strong independent predictor for birth weight and infant adiposity (6–10). Therefore, maternal blood TG level is essential in fetal growth and fat development.

A strong association of obesity and abnormal lipid metabolism is well-documented from humans to animal models. Maternal obesity, especially prepregnant obesity, has been identified as a key risk factor for many adverse pregnancy outcomes. However, our understanding of the impact of obesity on maternal adaptation in lipid metabolism to pregnancy is limited. Although increased fasting and postprandial blood TG concentrations have been observed in obesity-complicated pregnancy (7,9), studies have reported that obese women have a less pronounced rise in blood TG concentration during pregnancy (5,11–14). More interesting, our mouse study revealed that high-fat (HF) feeding during pregnancy significantly reduced maternal blood TG, despite the elevation of adiposity (15). Therefore,

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a systemic experiment was warranted to investigate the effects of obesity on the increase of maternal blood TG concentration during pregnancy, referred to as TG trajectory hereafter, and the underlying mechanism.

Lipoprotein lipase (LPL) is the primary driver of blood TG clearance. LPL activity is negatively regulated by several angiopoietin-like proteins (ANGPTL) (16–19). ANGPTL4 is the most potent LPL inhibitor in the ANGPTL family (17,20,21) and is highly expressed in white adipose tissue (WAT) and brown adipose tissue (BAT) (20,22). After secretion from cells, ANGPTL4 can be cleaved into an N-terminal domain and a COOH-terminal fibrinogen-like domain (23,24). The coiled-coil domain of the N-terminus inhibits LPL activity (25). ANGPTL4 expression is significantly increased during pregnancy (26–28).

Using mouse models, our current studies demonstrate that prepregnancy HF (ppHF) feeding induced maternal obesity. Compared with control (Con) dams, maternal blood TG concentrations were significantly lower in ppHF dams. Although ppHF feeding increased UCP1 expression, results from *Ucp1* gene knockout (*Ucp1*$^{-/-}$) mice ruled out the contribution of UCP1 in obesity-reduced maternal blood TG trajectory. Our study further revealed that ppHF feeding significantly increased postheparin blood LPL activity but showed no significant effect on hepatic TG secretion or postprandial intestinal TG absorption. Significantly, low expression levels of *Angptl4* were observed in the interscapular BAT (iBAT), gonadal WAT (gWAT), and livers of ppHF-fed dams. Pregnancy-induced increase of maternal blood TG concentration was almost abolished in *Angptl4* gene–deficient (*Angptl4*$^{-/-}$) dams. Importantly, overexpression of ANGPTL4 restored maternal blood TG concentrations of ppHF dams. Together, this study demonstrates that ANGPTL4 plays a crucial role in increasing maternal blood TG concentrations during pregnancy. Maternal obesity impairs the rise of maternal blood TG trajectory by reducing *Angptl4* expression in mice.

RESEARCH DESIGN AND METHODS

Materials

Antibody against human ANGPTL4 antibody was from R&D Systems (Minneapolis, MN). Anti-GAPDH and horseradish peroxidase–linked secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-UCP1 antibody was from Abcam (Cambridge, MA). TG assay kits were purchased from Wako Diagnostics (Richmond, VA). FBS, NuPAGE gels, SuperScript III Reverse Transcriptase, and oligo(dT)$_{12\text{-}18}$ primer were from Invitrogen (Carlsbad, CA). The LPL activity assay kit was from Cell Biolabs, Inc. (San Diego, CA). Ad-hANGPTL4 adenovirus was from Vector Biolabs (Malvern, PA). HF diet (60% kcal from fat, 20% kcal from protein, and 20% kcal from carbohydrate; energy density: 5.24 kcal/g) (catalog number D12492) and ingredient-matched low-fat diet (10% kcal from fat, 20% kcal from protein, and 70% kcal from carbohydrate; energy density: 3.85 kcal/g) (catalog number D12450J) were from Research Diets, Inc. (New Brunswick, NJ). Regular chow (17% kcal from fat, 25% kcal from protein, and 58% kcal from carbohydrate; energy density: 3.1 kcal/g) (catalog number 7912) was from Harlan Laboratories (Madison, WI).

Experimental Animals

C57BL/6 and *Ucp1*$^{-/-}$ mice were from The Jackson Laboratory (Bar Harbor, ME). *Angptl4*$^{-/-}$ mice were obtained from the Mutant Mouse Resource and Research Center and backcrossed for 10 generations into a C57BL/6 background (29,30). Some female C57BL/6 and *Ucp1*$^{-/-}$ mice were fed with HF or low-fat diet for 12 weeks and then regular chow 2 weeks before mating and during pregnancy. Sires were fed with chow. To avoid any effects from fetuses, *Ucp1*$^{-/-}$ and *Angptl4*$^{-/-}$ mice were crossed with wild-type (WT) mice to produce *Ucp*$^{+/-}$ or *Angptl4*$^{+/-}$ offspring. Pregnancy was determined by the presence of a vaginal plug and assigned the embryonic age (E)0.5. Maternal body composition was monitored by EchoMRI. Maternal blood samples were collected in the fed state at ~9:00 to 10:00 A.M. To ectopically overexpress ANGPTL4, 1×10^6 plaque-forming units of purified adenoviral vectors encoding human ANGPTL4 (Ad-hANGPTL4) or green fluorescent protein (Ad-Gfp) were injected through the tail vein into dams at E11.5, and samples were collected at E14.5 (31). Experiments using mouse models were carried out under the Association for Assessment and Accreditation of Laboratory Animal Care guidelines with approval from the University of California San Diego Animal Care and Use Committee.

Hepatic TG Release Rate Assay

Our previous study showed that maternal blood TG concentration peaks at E14.5 (31). Therefore, maternal hepatic TG secretion rates were measured at E14.5 after blocking endogenous LPL activity by intravenous injection of Poloxamer 407 (1,000 mg/kg) (BASF Corporation, Mount Olive, NJ). Mice were fasted overnight before injection. A series of blood samples were collected before and after Poloxamer 407 injection. Blood TG concentrations were measured using a Wako kit. The hepatic TG release rates were calculated by the accumulation of blood TG concentrations as we previously described (32).

Lipid Load Test

Pregnant mice (E14.5) were fasted overnight and orally fed with sunflower oil (0.4 mL). Blood samples were collected from the tail vein before and at 1, 2, 3, 4, and 5 h after gavage. To determine intestinal TG secretion in postprandial conditions, mice were injected with LPL inhibitor Poloxamer 407 10 min before gavage. Blood TG concentrations were determined, and increase rates were calculated.

Postheparin Plasma LPL Activity Assay

To release endothelial cell–attached LPL into blood, heparin (0.2 μ/g body weight) was injected into mice via the tail vein. Blood samples were collected 10 min after injection, and plasma samples were prepared. Postheparin

plasma LPL activity was determined using the fluorometric assay kit as we previously described (15).

Western Blot and Real-time PCR Assays

Protein samples were extracted from iBAT or other tissues and separated using NuPAGE gels. Proteins were blotted with the indicated antibodies (see details in figure legends). The bands from Western blots were quantified using Quantity One software (Bio-Rad). Total RNA was prepared from tissues using TRIzol. cDNA was synthesized using SuperScript III Reverse Transcriptase and oligo$(dT)_{12\text{-}18}$ primer. Real-time PCR was performed using a QuantStudio 3 Real-Time PCR System (Invitrogen) with specific primer pairs (Table 1). Expression data were normalized to the amount of β-actin.

Statistical Analysis

Data are expressed as a mean ± SEM. Statistical analyses were performed using the Student *t* test or ANOVA, followed by Bonferroni posttests using GraphPad Prism software. Differences were considered significant at $P < 0.05$.

Data and Resource Availability

The data sets and reagents generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

RESULTS

ppHF Feeding Impaired the Rise of Maternal Blood TG Concentrations During Pregnancy

During normal pregnancy, a significant elevation in maternal blood TG concentrations has been observed from humans to rodents. Surprisingly, our previous study observed that HF feeding during pregnancy significantly reduced maternal blood TG concentrations in C57BL/6 mice (15). To further study the effect of maternal obesity on maternal lipid metabolism, we fed C57BL/6 female mice with HF diet for 12 weeks prior to pregnancy. Mice were returned to regular chow 2 weeks before mating and during pregnancy to avoid any dietary effects during pregnancy. As expected, ppHF feeding significantly increased maternal body weight and fat mass through pregnancy (Fig. 1*A* and *B*). Significant increases in blood glucose and insulin concentrations were observed in ppHF mice before mating (Fig. 1*C* and *D*). Therefore, prolonged ppHF feeding induces maternal obesity.

Despite obesity and insulin resistance, blood TG concentrations of ppHF mice were comparable to those of Con dams before pregnancy (Fig. 1*E*, at E0.0). In Con dams, maternal blood TG concentrations steadily elevated and reached a peak at E14.5 in Fig. 1*E*. For ppHF dams, TG concentrations were significantly increased at E7.5 but were remarkably lower than those of Con dams at E14.5 and E18.5 (Fig. 1*E*). The areas under the blood TG concentration curve were also significantly less in ppHF dams (Fig. 1*F*). These results indicate that ppHF feeding impaired the increase of maternal blood TG concentration during pregnancy. Because all mice were fed with chow during pregnancy, these data indicate that ppHF-induced obesity, but not dietary fat, impairs the rise of maternal blood TG trajectory. Consistent with most recent rodent studies (33–40), a significant decrease in body weight was observed in fetuses from ppHF dams after E16.5 (Supplementary Fig. 1). Of note, maternal obesity usually increases birth weight in humans (5–9). Despite the opposite effects on fetal growth between rodents and humans, the parallel reduction in maternal blood TG concentrations and fetal

Table 1—Sequences for real-time PCR primers

Gene	Forward (5′ to 3′)	Reverse (5′ to 3′)
β-Actin	GGGGTGTTGAAGGTCTCAAA	CTGAACCCTAAGGCCAACC
Angptl4	GGGACCTTAACTGTGCCAAG	GAATGGCTACAGGTACCAAACC
Angptl3	GGAGCAGCTAACCAACTTAATTCT	TGTTGTCTTGCTGTTCTACAAAACT
Angptl8	GCCACACAAGAATTTGAGAC	GCCAGTGAGAGCCCATAAGA
Scd1	GTGGGCAGGATGAAGCAC	AGCTGGTGATGTTCCAGAGG
Apob	TTCTTCTCTGGAGGGGACTG	GGCACTGTGGGTCTGGAT
Gpihbp1	TGTCCTCCTGATCTTGCTACTA	TCTCCTCCTCTTCCTCTTCATC
Srebp1c	CTGTCTCACCCCCAGCATAG	GAACTGGACACAGCGGTTTT
Pparγ1	CTGTGTCAACCATGGTAATTTCTT	TGCTGTTATGGGTGAAACTCTG
Pparγ2	ATGCACTGCCTATGAGCACT	CAACTGTGGTAAAGGGCTTG
Fasn	ACTCCACAGGTGGGAACAAG	CCCTTGATGAAGAGGGATCA
Vldlr	CCTATAACTAGGTCTTTGCAGATATGG	GAGCCCCTGAAGGAATGCC
LPL	GAAGTCTGACCAATAAGAAGGTCAA	TGTGTGTAAGACATCTACAAAATCAGC
CD36	TTGAAAAGTCTCGGACATTGAG	TCAGATCCGAACACAGCGTA
Mtp	TGAGAGGCCAGTTGTGTGAC	GGCAGTGCTTTTTCTCTGCT
Pepck	TCGATGCCTTCCCAGTAAAC	CTGGCACCTCAGTGAAGACA
Ucp1	TAAGCCGGCTGAGATCTTGT	GGCCTCTACGACTCAGTCCA

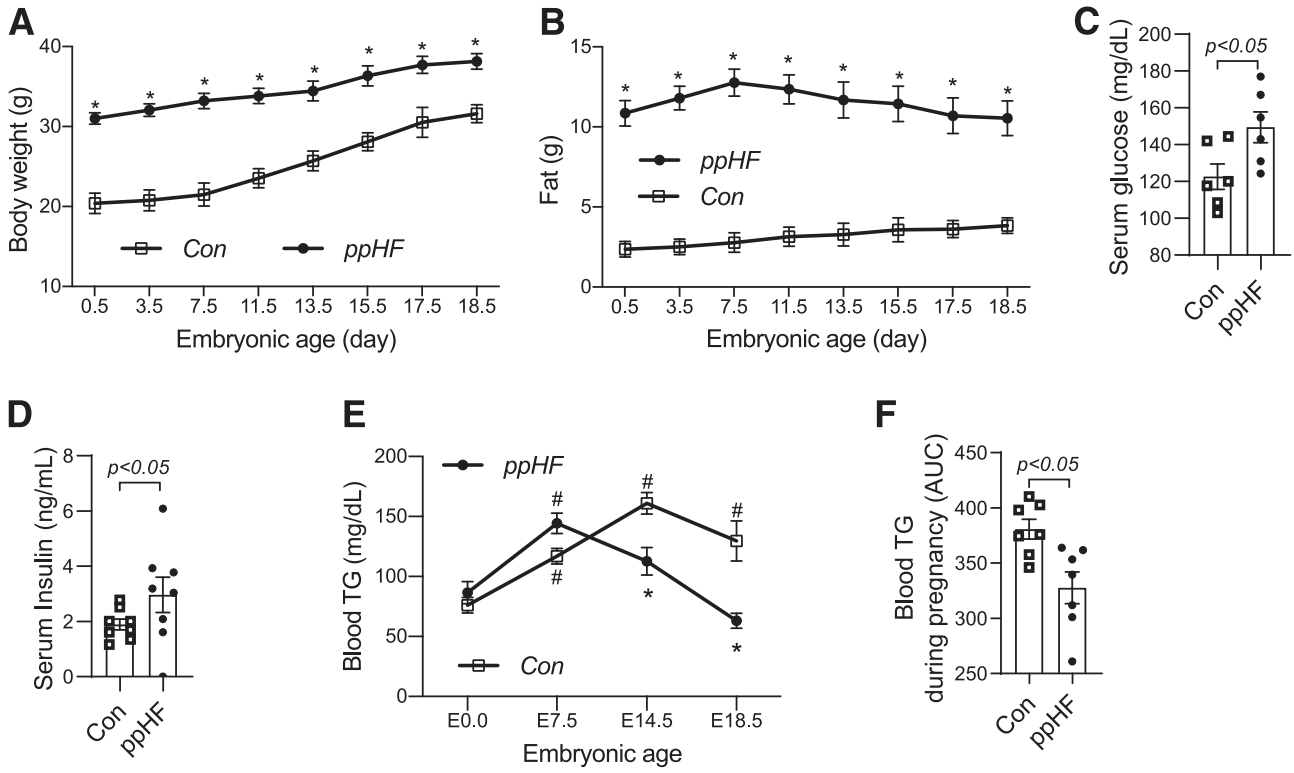

Figure 1—ppHF induced maternal obesity and reduced maternal blood TG concentration. C57BL/6 female mice were fed with HF diet for 12 weeks and then returned to regular chow 2 weeks before mating and during pregnancy. Body weight (*A*) and fat tissue mass (*B*) were monitored using EchoMRI. Increased blood glucose (*C*) and insulin (*D*) concentrations were observed in ppHF-fed mice before pregnancy in the fed state. *E*: Blood TG concentrations were monitored during pregnancy. *F*: The area under the blood TG curve (AUC) (*E*) was calculated. Data are presented as mean ± SEM; $n = 8$–12 or shown in the bar graph. *$P < 0.05$ vs. samples from Con dams; #$P < 0.05$ vs. samples from the same group at E0.0.

body weights of ppHF mice supports the notion that maternal TG plays an important role in fetal growth (6–10). A separate project is studying how obesity-impaired maternal TG metabolism induces intrauterine growth restriction. The current study focuses on the mechanisms underlying the obesity-impaired rise of maternal blood TG trajectory.

ppHF Feeding Increased Systemic LPL Activity and Exhibited No Significant Effect on Maternal Hepatic TG Release or Postprandial Intestinal TG Secretion Rates

Blood TG concentration is mainly determined by the counteraction of hepatic and intestinal TG secretion and TG clearance at peripheral tissues. To study the effect of ppHF on postprandial TG metabolism, we first performed an oil load test. As previously reported for HF-fed nonpregnant mice (41), significantly decreased blood TG concentrations were observed during the oil load test in ppHF dams compared with Con mice (Fig. 2*A*). Next, we measured maternal hepatic TG secretion rate during fasting conditions, a time period that excludes the contribution of postprandial intestinal TG secretion. As showed in Fig. 2*B*, similar hepatic TG release rates were observed between ppHF and Con dams, indicating that ppHF feeding did not alter maternal hepatic TG secretion during pregnancy. Although we observed no differences in maternal liver weight between ppHF and Con dams (Fig. 2*C*), there was a significant increase in hepatic TG content in ppHF dams (Fig. 2*D*). There were no differences between ppHF and Con dams in the hepatic mRNA levels of *Srebp1c*, *Fasn*, and *Dgat1*, but we observe a significant increase in *Cd36* and a reduction in *Mtp* in ppHF dams (Fig. 2*E*). These results suggest that ppHF feeding significantly enhanced hepatic TG accumulation during pregnancy. Further studies will be required to determine whether de novo lipogenesis and/or lipoprotein particle release contribute to the increased hepatic TG accumulation in ppHF dams.

We then measured postprandial intestinal TG secretion by an oil load test after inhibition of systemic LPL activity. Interestingly, after blocking systemic LPL activity with Poloxamer 407, the increase of blood TG concentrations during an oil load test was similar between ppHF and Con dams (Fig. 2*F*), indicating ppHF and Con dams had comparable postprandial intestinal TG secretion rates during pregnancy. These results of oil load tests (with or without LPL inhibition) and hepatic TG production assays suggest that TG clearance is the primary contributor to ppHF-impaired maternal blood TG trajectory.

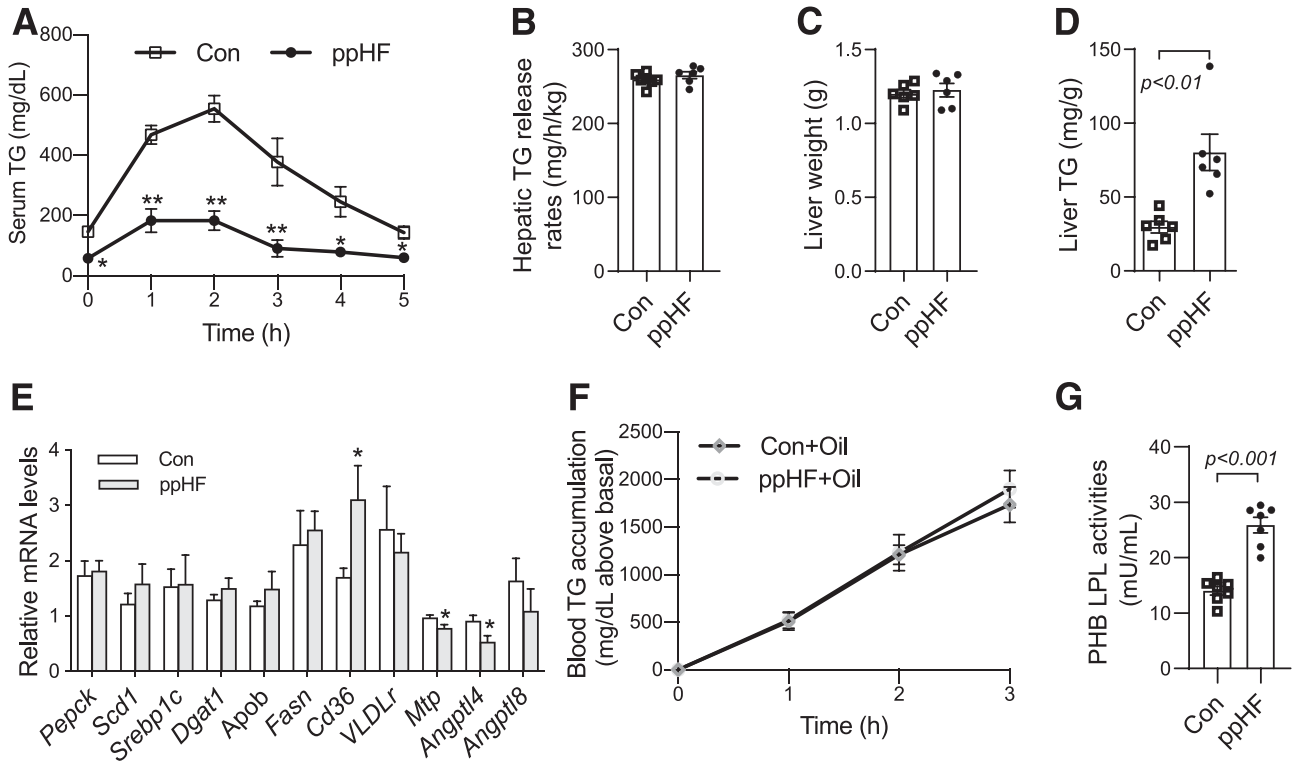

Figure 2—ppHF increased maternal blood LPL activity but showed no effect on hepatic and intestinal TG release rates. *A*: Blood TG concentrations were measured after an oil gavage. *B*: Hepatic TG release rates were determined by measuring blood TG concentrations after injection of LPL inhibitor Poloxamer 407 in fasted mice. *C*: The liver tissue weight was measured by a scale. *D*: Liver TG content was determined by extracting TG from homogenized tissue. *E*: Levels of target mRNAs were measured by real-time PCR. *F*: Blood TG concentrations were measured after oil load and LPL inhibition (E14.5). *G*: Postheparin blood (PHB) LPL activity was measured using maternal samples at E14.5. Data are presented as mean ± SEM; n = 6–8 or shown in bar graph. $^*P < 0.05$, $^{**}P < 0.001$ vs. Con dams.

LPL-catalyzed TG hydrolysis plays a predominant role in blood TG clearance. We measured postheparin blood LPL activity to assay the effect of ppHF feeding on maternal systemic TG clearance. As shown in Fig. 2*G*, postheparin blood LPL activity was significantly increased in ppHF dams, indicating that maternal obesity increases blood TG clearance. Together, these results indicate that ppHF feeding impairs the increase of maternal blood TG concentrations during pregnancy, mainly through increasing LPL activity and blood TG clearance.

Maternal Obesity Increased UCP1 Expression, but the *Ucp1* Gene Knockout Did Not Attenuate the Inhibitory Effects of ppHF on Maternal Blood TG Trajectory

It has been reported that BAT activation plays an important role in blood TG clearance (42). UCP1 protein levels were significantly increased in iBAT from ppHF dams (Fig. 3*A*). Our recent study showed that HF feeding increased BAT activation and energy expenditure in pregnant mice (43). These results prompted us to speculate that increased UCP1 expression and BAT activation may contribute to increased blood TG clearance and the reduction of maternal blood TG concentrations in ppHF dams. To verify this hypothesis, we fed $Ucp1^{-/-}$ female mice with HF diet using the same regimen for the C57BL/6 mice. Like WT Con mice, maternal blood TG concentrations of chow-fed $Ucp1^{-/-}$ dams (Con[$Ucp1^{-/-}$]) increased significantly during pregnancy (Fig. 3*B*). Interestingly, there was no significant difference in blood TG concentrations between $Ucp1^{-/-}$ and WT mice before or during pregnancy (Fig. 3*B* and *C*). Most importantly, a similar reduction of maternal blood TG trajectory was detected in ppHF-fed WT and $Ucp1^{-/-}$ dams (Fig. 3*B* and *C*). These results refute the hypothesis that increased UCP1 expression mediates obesity-enhanced blood TG clearance and attenuated increase of maternal blood TG.

ppHF Feeding Reduced *Angptl4* Gene Expression, and Overexpression of ANGPTL4 in ppHF Dams Restored Maternal TG Concentration

During normal pregnancy, *LPL* gene expression was significantly reduced in both gWAT and iBAT (Fig. 4*A* and *B*). Despite the increased blood LPL activity of ppHF dams (Fig. 2*G*), LPL expression levels were not significantly altered in gWAT, iBAT (Fig. 4*A* and *B*), skeletal muscle, and hearts (Supplementary Fig. 2*A* and *B*) compared with controls. Similar to the study of HF feeding during pregnancy (15), *LPL* mRNA levels were significantly increased in the placentas of ppHF dams (Supplementary Fig. 2*C*).

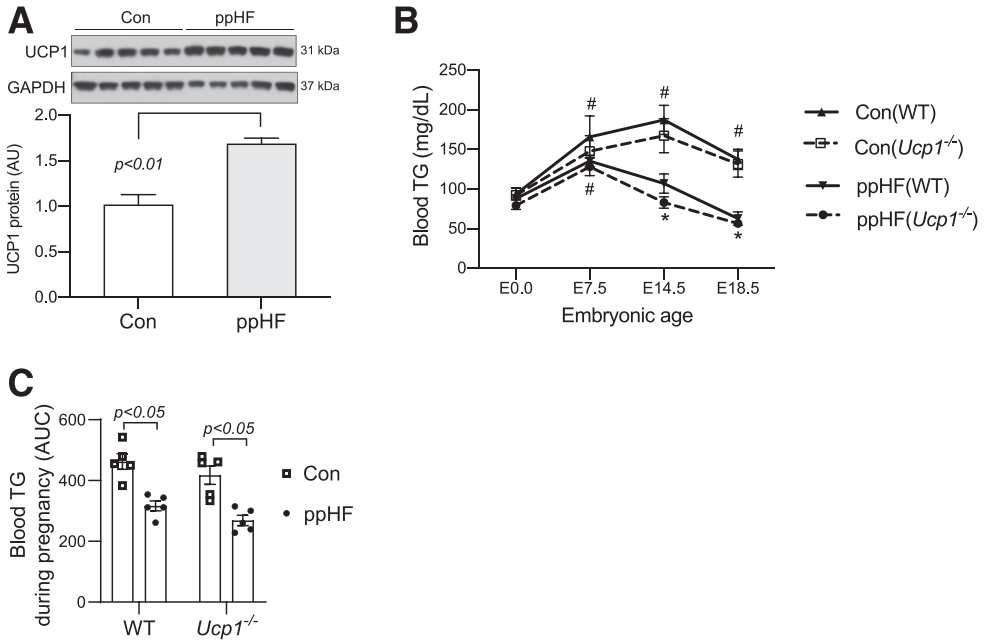

Figure 3—ppHF increased UCP1 expression, but *Ucp1*$^{-/-}$ mice exhibited no significant change in blood TG concentrations during pregnancy. *Ucp1*$^{-/-}$ and WT female mice were fed with HF diet for 12 weeks and then returned to regular chow 2 weeks before mating and during pregnancy. *A*: UCP1 protein levels were measured by Western blotting using iBAT from Con and ppHF WT dams (E18.5; $n = 14$). *B*: Blood TG concentrations of both chow- or ppHF-fed WT and *Ucp1*$^{-/-}$ dams were monitored during pregnancy. *C*: The area under the curve (AUC) of blood TG concentrations was compared between Con and ppHF dams. Data are presented as mean ± SEM. *$P < 0.05$ vs. Con dams (WT or *Ucp1*$^{-/-}$); #$P < 0.05$ vs. samples from the same group at E0.0. AU, arbitrary units.

LPL activity is negatively regulated by a group of ANGPTL proteins, including ANGPTL3, ANGPTL4, and ANGPTL8. ANGPTL4 is the most potent LPL inhibitor and mainly expressed in WAT, BAT, and the liver (20,22). In line with previous reports (26–28), *Angptl4* mRNA levels were robustly increased in both gWAT (Fig. 4*C*) and iBAT (Fig. 4*D*), but not livers (Supplementary Fig. 2*D*), during pregnancy. Of note, due to the lack of a specific antibody to mouse ANGPTL4, only ANGPTL mRNA levels were measured. To determine if ANGPTL4 plays a role in pregnancy-induced hypertriglyceridemia, we measured the maternal blood TG trajectory of *Angptl4* gene knockout (*Angptl4*$^{-/-}$) mice. Interestingly, although there was no significant difference in blood TG concentrations before pregnancy between *Angptl4*$^{-/-}$ and WT mice, the increases in maternal blood TG levels were almost abolished in *Angptl4*$^{-/-}$ dams (Fig. 4*E* and *F*). Together, these results indicate that ANGPTL4 plays a critical role in increasing maternal blood TG concentrations during pregnancy.

Comparing mRNA levels for lipid metabolism genes in fat and livers, we found a significant reduction of *Angptl4* mRNA levels in gWAT, iBAT, and liver of ppHF dams (Figs. 2*E* and 4*G* and *H* and Supplementary Fig. 3*A*). To study the role of decreased Angptl4 in ppHF feeding–reduced maternal blood TG trajectory, an adenovirus-mediated in vivo hepatic transduction approach was used to ectopically express human ANGPTL4 protein in pregnant mice (31) (Supplementary Fig. 3*B*). In line with studies of nonpregnant mice (24), maternal blood TG concentrations were significantly increased in Ad-hANGPTL4–treated pregnant mice (Fig. 4*I*). Most importantly, the reduction of maternal blood TG concentrations in ppHF dams was attenuated by ANGPTL4 expression (Fig. 4*I*). Together, these results indicate that decreased *Angptl4* expression underlies the obesity-impaired rise of maternal blood TG trajectory during pregnancy.

DISCUSSION

TG-rich lipoprotein particles are essential vehicles for transporting maternal lipids to the placenta and fetal compartment. Through a series of coordinated maternal metabolic adaptation, maternal blood TG concentration steadily increases to a significantly high level to ensure fetal lipid supply. Therefore, in addition to glucose and amino acids, maintaining physiological hypertriglyceridemia in maternal circulation is vital for fetal development and growth (1,6,10). Using genetic mouse models, our study unveiled a critical role of ANGPTL4 in increasing maternal blood TG trajectory during pregnancy. The remarkable reduction in maternal blood TG concentrations in ppHF dams indicated that obesity impairs the rise of maternal blood TG trajectory during pregnancy in mice. Our current studies further demonstrated that the reduction of ANGPTL4 expression serves as an underlying mechanism of obesity-impaired maternal blood TG trajectory.

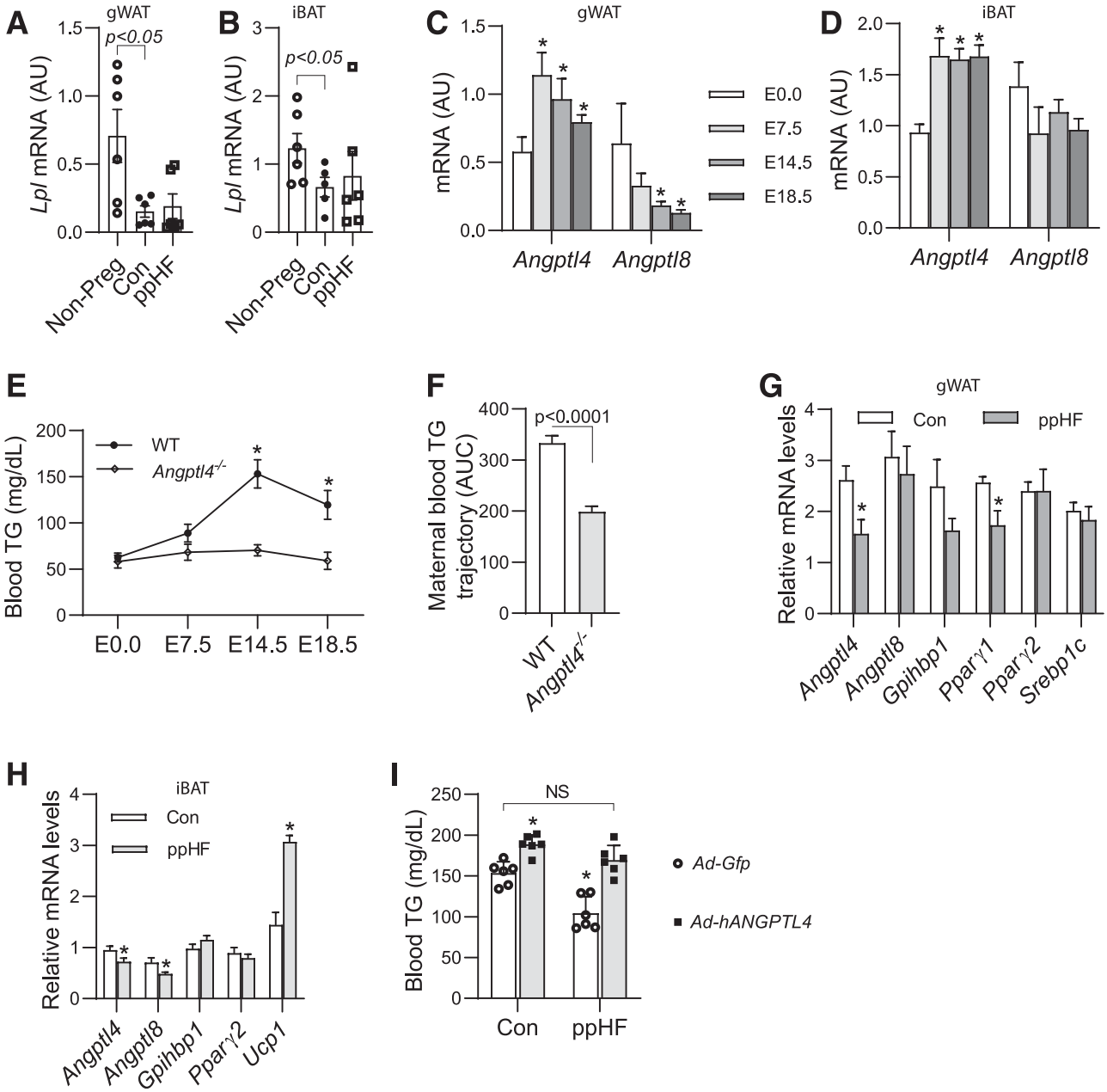

Figure 4—*Angptl4* gene deletion impaired the rise in maternal blood TG levels, and overexpression of ANGPTL4 restored maternal blood TG concentrations in ppHF dams. *A* and *B*: *Lpl* mRNA levels were measured by real-time PCR using gWAT and iBAT samples from nonpregnant C57BL/6 mice (Non-Preg), Con, and ppHF dams (E18.5). *C* and *D*: gWAT and iBAT tissue samples were collected from C57BL/6 dams at indicated embryonic ages. mRNA levels of *Angptl4* and *Angptl8* were determined by real-time PCR. *E* and *F*: WT and *Angptl4*$^{-/-}$ mice were crossed to produce *Angptl4*$^{-/+}$ fetuses, which ensured the same genotype of fetuses. *E*: Maternal blood TG concentrations were measured during pregnancy. *F*: The area under the curve (AUC) of maternal blood TG was compared. *G* and *H*: mRNA levels of indicated genes were measured by real-time PCR using gWAT and iBAT samples from Con and ppHF dams. *I*: Three days after Ad-GFP or Ad-hANGPTL4 injection, maternal blood TG concentrations were measured in Con and ppHF dams. Data are presented as mean ± SEM; n = 11–16 or shown in bar graph. *$P < 0.05$ vs. E0.0, WT, or Con dams. AU, arbitrary units.

In addition to increasing hepatic and intestinal TG production, reduction of LPL-mediated TG clearance at peripheral tissues is a main driving force that increases maternal blood TG concentrations, especially in late pregnancy. A significant reduction in postheparin plasma LPL activity and decreased LPL expression were observed in pregnant women and rats (2,44,45). Similarly, our data indeed reveal a robust reduction in LPL expression in maternal metabolically active tissues. In addition, the expression of LPL inhibitor ANGPTL4 is also significantly increased during pregnancy (26–28,46) (Fig. 4*B* and *C*). These data promote a logical assumption that

decreased LPL expression and increased ANGPTL4 should both contribute to the decrease of maternal blood TG clearance and subsequent increase in blood TG concentrations during normal pregnancy. Surprisingly, almost no increase in maternal blood TG concentration was observed in *Angptl4*$^{-/-}$ dams (Fig. 4*E* and *F*). Such robust effects of *Angptl4* deficiency on maternal blood TG concentrations demonstrate a predominant role of ANGPTL4 in raising maternal blood TG concentrations during pregnancy. ANGPTL3 and ANGPTL8 are two other members of the ANGPTL family. ANGPTL3 is mainly expressed in the livers (47), while ANGPTL8 is expressed in the liver and fat of mice but exclusively in the livers of humans (48–50). In contrast to the increased Angptl4 expression during pregnancy, *Angptl8* mRNA levels were significantly reduced in gWAT (Fig. 4*C*) and trend to decrease in livers of healthy dams (Supplementary Fig. 2*D*). No change in Angptl3 expression was detected in dams' livers (Supplementary Fig. 2*D*). Although our studies cannot completely rule out the involvement of ANGPTL3 and ANGPTL8, these gene expression data suggest a minor role of ANGPTL3 and ANGPTL8 in regulating maternal TG trajectory during pregnancy. Together, our data indicate that ANGPTL4 is a key player in controlling maternal blood TG metabolism during pregnancy.

ANGPTL4 is the most potent LPL inhibitor among the ANGPTL family (16–22). ANGPTL4 gene mutation or knockout induced hypotriglyceridemia (17,51). Although we did not measure blood ANGPTL4 protein levels due to the lack of a specific antibody, the significant reduction in *Angptl4* expression and maternal blood TG concentrations of ppHD-fed dams confirms the inhibitory effect of ANGPTL4 on LPL. The restoration of maternal blood TG concentrations of ppHF dams by ectopic ANGPTL4 overexpression further confirms the causal role of decreased *Angptl4* expression in obesity-reduced maternal blood TG concentrations. Although ANGPTL4 can act as an endocrine factor, a study has reported that ANGPTL4 inhibits LPL activities also through a paracrine manner (19). Our overexpression approach used the endocrine feature of this protein. Future studies are required to investigate if ANGPTL4 acts in a paracrine or a systemic manner to increase maternal TG concentrations. For example, both ppHF and HF feeding during pregnancy increase LPL gene expression in placentas (15) (Supplementary Fig. 2*C*). It will be interesting to know if decreased maternal ANGPTL4 also contributes to increased LPL activity at the placenta through an endocrine effect.

Obesity, insulin resistance, and hyperlipidemia are closely associated components of the metabolic syndrome. Although few studies have reported that maternal obesity is associated with a low magnitude of pregnancy-induced increase in maternal blood TG concentrations (5,11–14), most clinical data suggest a positive correlation between maternal adiposity and blood TG levels (1,4–6,8–10). These human studies support the notion that obesity increases maternal blood TG concentrations. However, there is no direct experimental evidence indicating the causal relationship between obesity and hypertriglyceridemia. In contrast, despite obesity, a significant reduction in blood TG concentrations was observed in pregnant mice (15) (Fig. 1*E* and *F*). By avoiding dietary effects during pregnancy, the current study further demonstrated that prepregnant obesity impairs the rise of maternal blood TG trajectory in mice. Together, these data indicate that maternal obesity reduces the increase of maternal blood TG concentrations in mice. Unfortunately, our study does not provide any evidence to explain these opposite metabolic phenotypes between humans and mice. However, similar to most rodent studies (33–40), a significant reduction in body weight was observed in fetuses from ppHF dams, indicating a parallel change in maternal blood TG concentration and fetal growth. In humans, maternal blood TG concentrations were also associated with birth weight (1,6,10). In other words, the positive association between maternal blood TG concentrations and fetal growth is conserved from mice to humans. Therefore, obese pregnant mice are still valuable models that will help us to understand how obesity alters maternal lipid metabolism. As with most animal studies, caution should be taken when applying the mechanistic information from animal work to humans. As with humans, maternal obesity induces insulin resistance in mice (Fig. 1*C* and *D*). Surprisingly, the apparent insulin resistance in ppHF dams accompanied a reduction in blood TG levels. Therefore, studies are required to determine the role of insulin resistance and its relationship with ANGPTL4 in obesity-impaired maternal TG metabolism.

In summary, this study demonstrates that obesity impairs the rise of maternal blood TG concentration during mouse pregnancy. ANGPTL4 plays a crucial role in increasing maternal blood TG concentrations. Obesity reduces ANGPTL4 expression, leading to the reduction of maternal blood TG trajectory in obese mice.

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