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Prolonged Prepregnant Maternal High-Fat Feeding Reduces Fetal and Neonatal Blood Glucose Concentrations by Enhancing Fetal β-Cell Development in C57BL/6 Mice

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OBESITY STUDIES

The main objective of this study was to investigate the effect of maternal obesity on offspring's glucose metabolism during the perinatal period. Maternal obesity was established by feeding C57BL/6 mice with a high-fat (HF) diet before or during pregnancy. Our results showed that prolonged prepregnant HF feeding but not HF feeding during pregnancy significantly reduced fetal and neonatal blood glucose concentrations. Remarkably, elevated blood insulin concentrations and increased activation of insulin signaling were observed in fetuses and neonates from prepregnant HF-fed dams. In addition, significantly larger β-cell areas were observed in pancreases of fetuses and neonates from prepregnant HF-fed dams. Although there was no significant change in placental cross-sectional area or GLUT 1 expression, prepregnant HF feeding significantly enhanced the expression of genes that control placental fatty acid supply. Interestingly, reducing fatty acid supply to the placenta and fetus by placental-specific knockout of adipose triglyceride lipase not only reduced fetal β-cell area and blood insulin concentration but also attenuated prepregnant HF feeding–induced reduction in offspring blood glucose concentrations during the perinatal period. Together, these results indicate that placental and fetal fatty acid supply plays an important role in fetal β-cell development, insulin secretion, and glucose metabolism. Prolonged prepregnant maternal HF feeding resembles pregravid maternal obesity in mice, which reduces fetal and neonatal blood glucose concentrations by enhancing fetal β-cell development and insulin secretion.

Compelling clinical data have demonstrated that maternal obesity closely associates with neonatal hypoglycemia (1–7). Most importantly, maternal BMI predicts neonatal hypoglycemia independent of maternal glucose concentrations, indicating that maternal obesity itself imposes a major risk for neonatal hypoglycemia (1,8,9). If not appropriately recognized and successfully treated, progressive and recurrent severe neonatal hypoglycemia can cause both acute physiological decompensation and serious long-term brain damage (9–13). Therefore, owing to the obesity epidemic, identifying the effect and the mechanisms underlying maternal obesity–induced neonatal hypoglycemia could lead to diagnostic, preventive, and therapeutic strategies that might significantly improve pregnancy outcomes.

Congenital hyperinsulinemic hypoglycemia in diabetic pregnancies suggested the corollary hypothesis that maternal obesity increases fetal glucose supply, which in turn stimulates fetal β-cell and islet development and insulin secretion that induces neonatal hypoglycemia. Unfortunately, there are insufficient human experimental data to verify this hypothesis. A human study even reported that hyperinsulinemia was not present in the infants who were born to obese mothers and suffered neonatal hypoglycemia (4). We therefore performed the study reported here in which prepregnant and pregnant mice were fed a high-fat (HF) diet to produce maternal obesity to investigate whether and how maternal obesity affects fetal and neonatal pancreatic development, insulin secretion, and glucose metabolism.

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By comparing fetuses and neonates from two obese dam models, this study revealed that prolonged prepregnant HF feeding significantly reduced blood glucose concentrations not only in neonates but also in fetuses in late pregnancy. In addition to the reduced blood glucose concentrations, prolonged prepregnant HF feeding significantly increased fetal and neonatal blood insulin concentrations and β-cell mass. Hyperactivation of insulin signaling also was detected in metabolically active tissues, indicating that excessive insulin production was responsible for the prepregnant HF feeding–induced reduction of perinatal blood glucose concentrations. By using a line of mice with placental-specific knockout of the adipose triglyceride (TG) lipase (*Atgl*) gene, our study further demonstrated that placental and fetal fatty acid (FA) supply plays an important role in pancreatic β-cell and islet development and might mediate prepregnant HF feeding–induced fetal and neonatal hyperinsulinemia and hypoglycemia.

RESEARCH DESIGN AND METHODS

Materials

Antibodies against AKT and phospho-AKT (Ser473) were from Cell Signaling (Danvers, MA). Anti-GAPDH and horseradish peroxidase–linked secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Glucose, glucose oxidase, collagenase, and RPMI medium were from Sigma-Aldrich (St. Louis, MO). Free FA (FFA) and TG assay kits ware purchased from Wako Diagnostics (Richmond, VA). The LipidTOX green neutral lipid stain, NuPAGE gels, SuperScript III reverse transcriptase, and oligo(dT)$_{12–18}$ primer were from Invitrogen (Carlsbad, CA). The ultrasensitive mouse insulin ELISA kit was from ALPCO (Salem, NH). The anti-insulin and anti-glucagon antibodies were from Abcam (Cambridge, MA). HF diet (60 kcal% from fat; catalog number D12492) was from Research Diets (New Brunswick, NJ). The control diet (CD) (17 kcal% from fat, catalog number 7912) was from Harlan Laboratories (Madison, WI).

Experimental Animals

C57BL/6J and *Atgl*$^{f/f}$ mice were from The Jackson Laboratory (Bar Harbor, ME). The *Tpbpa-Cre* mice were from the Canadian Mouse Mutant Repository and were backcrossed to C57BL/6 for seven generations in our laboratory (14). C57BL/6J female mice (10 weeks old) were randomly assigned to three groups: *1*) HF fed for 3 months then switched back to CD (named as prepregnant obese dam, POD), *2*) HF-fed only during pregnancy to resemble excessive weight gain (named as EWGD), and *3*) CD-fed during the entire study (control dam, ConD). The *Tpbpa-Cre* and *Atgl*$^{f/f}$ mice were crossed to produce *Atgl*$^{f/f}$*;Tpbpa-Cre*$^{+/-}$ breeders. The *Atgl*$^{f/f}$*;Tpbpa-Cre*$^{+/-}$ female mice were prepregnantly fed the HF diet or CD as above described for C57BL/6 mice. The *Atgl*$^{f/f}$*;Tpbpa-Cre*$^{+/-}$ (pAtglko) and *Atgl*$^{f/f}$*;Tpbpa-Cre*$^{-}$ (Con) littermates were produced by mating *Atgl*$^{f/f}$*;Tpbpa-Cre*$^{+/-}$ breeders (Fig. 5*B*). Pregnancy was determined by the presence of a vaginal plug and assigned the embryonic age E0.5. Placentas and fetuses were collected through cesarean section at E18.5 at fed state. The first day of the presence of a new litter was assigned as postnatal 1 (P1). Neonatal tissue samples were collected at the fed state also.

To study insulin-stimulated glucose metabolism and signaling, regular insulin (0.75 units/kg in 20 μL saline) or saline was intraperitoneally injected into placenta-attached fetuses (E18.5) and neonates (P1). Tissue samples were collected 10 min later under a dissection microscope. Fetal and neonatal blood samples were collected through the neck incision during decapitation. Except for insulin-treated fetuses, fetal blood was collected immediately after the placental cord was cut. Serum samples were prepared from both maternal and fetal blood samples and used for hormone and metabolites assay. Body compositions of adult mice were determined using an EchoMRI System (Houston, TX). Genotype and sex of fetuses and neonates were determined by PCR (15). Experiments using mouse models were performed 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.

Histology, Immunohistology, Immunofluorescence, Placenta Cross-Sectional Surface Area, and β-Islet Area Assay

Placentas and pancreases were fixed in 4% paraformaldehyde and embedded in optimal cutting temperature compound. Sections with placentas were stained with hematoxylin and eosin and scanned using a microscope (BZ-X800E; Keyence, Laguna Hills, CA). The labyrinth zone, junction zone, and the decidua basalis of the placenta were manually selected (16). The lipid droplets in placentas were stained using the LipidTOX green neutral lipid stain reagent. The β-cells in the pancreatic sections were stained by immunohistology and immunofluorescence with an antibody against insulin (16). The areas of placental zones and β-cells were measured using Keyence BZ-X Analyzer software. The ratio (%) of each area was calculated by dividing each area of the placental zone or insulin-positive cell area by the total area of the placenta or pancreas.

Pancreatic Islet Isolation and Insulin Secretion Assay

Pancreases were collected at E18.5 or P1. Pancreatic islets were isolated by collagenase digestion and gradient centrifugation through polysucrose and sodium diatrizoate at a density of 1.077 g/mL using a previously described protocol (17). Handpicked size-matched islets (10 per well) were incubated in RPMI medium for 2 h. The medium was switched to Krebs-Ringer solution with 2.8 mmol/L and then 20 mmol/L glucose. Medium samples were collected after incubation for 1 h at 37 °C. Insulin in Krebs-Ringer solution and in the β-cells was measured using an ELISA kit.

Western Blot and Real-Time PCR Assays

Protein samples were extracted from placentas, livers, or skeletal muscles 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 following the manufacturer's protocol. cDNA was synthesized using SuperScript III Reverse Transcriptase and oligo(dT)$_{12–18}$ primer. Real-time PCR was performed using an Mx3000p real-time PCR system (Stratagene, San Diego, CA) and specific primers (Table 1). The levels of PCR product were calculated from standard curves established from each primer pair. Expression data were normalized to the amount of GAPDH.

Statistical Analysis

Data are expressed as the 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 $P < 0.05$.

RESULTS

Prepregnant HF Feeding Reduced Fetal and Neonatal Blood Glucose Concentrations

Two dietary regimens were used to create maternal obesity models. To reproduce pregravid obesity and avoid any dietary effect during pregnancy, one group of C57BL/6 female mice was fed the HF diet for 12 weeks then switched back to the CD 2 months before mating. Prepregnant HF feeding significantly increased body weight and adiposity of C57BL/6 female mice (named as POD) (Fig. 1*A* and *B*). Despite a reduction of body weight and adiposity after switching to the CD, the POD mice still maintained significantly higher levels of body fat before and during pregnancy (Fig. 1*B* and *C*). These data indicate that prolonged prepregnant HF feeding created a mouse model with pregravid obesity. To resemble excessive weight gain (EWG) during pregnancy, one group of age-matched female mice were fed the HF diet only during pregnancy (EWGD). As expected, the EWGDs gained significantly more body fat than the ConD mice during most time points of pregnancy (Fig. 1*C*). There was no significant difference in litter size (Supplementary Fig. 1*A*). Consistent with other rodent studies (18–20), body weights of fetuses from PODs and EWGDs were markedly lower than those of fetuses from CD-fed dams (Fig. 1*D*).

Blood glucose concentrations of both EWGDs and PODs were comparable to ConDs at the end of pregnancy (Fig. 1*E*), although PODs exhibited a trend of increase during pregnancy (Supplementary Fig. 1*B*) ($P > 0.05$). Similarly (21), maternal HF feeding significantly increased maternal blood insulin concentrations (Fig. 1*F*) but not maternal blood FFA concentrations (Fig. 1*G*). These results indicate that HF feeding before or during pregnancy increased maternal adiposity but did not significantly increase the maternal blood glucose concentration. Uniquely, unlike maternal blood glucose, significant reductions in blood glucose concentrations were detected in both male and female fetuses (E18.5) and neonates (P1) from PODs but not EWGDs (Fig. 1*H*). As we previously reported (21), there was a significant increase in blood FFA but not TG concentrations in fetuses from EWGDs and PODs (Fig. 1*I* and *J*). Together, these results indicate that prolonged prepregnant maternal HF feeding reduces fetal and neonatal blood glucose concentrations.

Of note, under physiological conditions, blood glucose concentrations of human neonates are significantly lower than those in adults (22). Using C57BL/6 mice, our study showed that fetal and neonatal blood glucose concentrations were ~40–60 mg/dL before P3 (Fig. 1*K*), indicating that similar to humans, fetal and neonatal mouse blood glucose concentrations are maintained at relatively lower levels than in adults. Because a sex-dependent difference in blood glucose concentration was not observed (Fig. 1*H*), the remainder of the data reported include males and females combined. To focus on pregravid obesity and avoid dietary effects during pregnancy, only the offspring of ConD (ConD-OS) or POD (POD-OS) were compared in the following studies. In addition, fetuses (E18.5) and neonates (P1) were studied in the following studies to focus on perinatal glucose metabolism.

Prepregnant HF Feeding Exhibited No Significant Effect on Placental Structure and Expression of GLUT1

The transplacental glucose gradient is the main driving force for transporting glucose from maternal blood to the fetal circulation. The reduction in fetal blood glucose concentrations and the normal PODs' maternal blood glucose levels (Fig. 1*E* and *H*) demonstrated an increased transplacental glucose gradient. To rule out a failure of maternal-to-fetal glucose transport by the placenta as a mechanism underlying the decreased fetal blood glucose concentrations of PODs, we conducted the following studies to determine the effect of prepregnant HF feeding on placental structure and the expression of GLUT1, the principal GLUT in the placental trophoblast. The placental weights (Fig. 2*A*), placental cross-sectional area (Fig. 2*B*), and GLUT1 protein levels of POD-OS (Fig. 2*C*) were comparable to ConD-OS. Although the current study did not measure the placental glucose transport rate, the above data do not support a causal role of placental glucose transport in prolonged prepregnant HF feeding–induced

Table 1—Sequences for real-time PCR primers

Gene	Forward (5′ to 3′)	Reverse (5′ to 3′)
18S rRNA	CGAAAGCATTTGCCAAGAAT	AGTCGGCATCGTTTATGGTC
Glut1	GACCCTGCACCTCATTGG	GATGCTCAGATAGGACATCCAAG

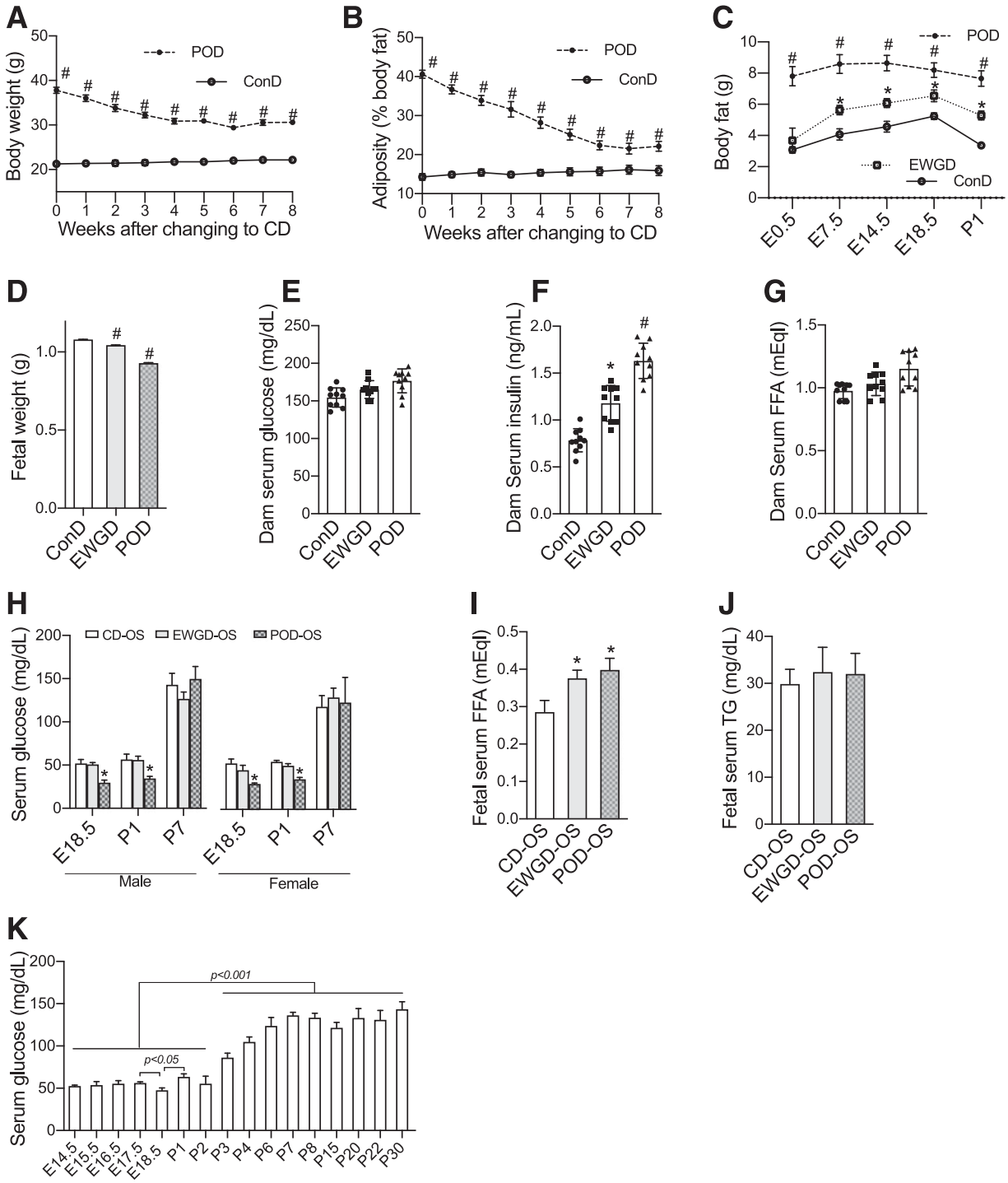

Figure 1—Prepregnant HF feeding increased maternal adiposity and reduced fetal and neonatal blood glucose concentrations. *A–C*: C57BL/6 female mice (10 weeks old) were randomly selected and fed the HF diet for 12 weeks and then switched back to the CD. *C*: To resemble EWG, one group of age-matched mice were fed the HF diet only during pregnancy. Body weight (*A*) and adiposity (*B*) were monitored weekly after changing to the CD and during pregnancy (*C*). *D*: Fetal body weights were measured at E18.5. Dam serum glucose (*E*), insulin (*F*), and FFA (*G*) concentrations were determined using samples at fed state of E18.5. *H–J*: Offspring blood samples were collected at fed state. Fetal serum samples were used for FFA (*I*) and TG assay (*J*) at E18.5. *K*: The C57/BL/6 dams (10–14 weeks old) were fed the CD. n = 10–14 for dams (*A–G*), n = 46–62 for fetuses (*H–K*), and n = 16–22 neonates. #$P < 0.01$ or *$P < 0.05$ vs. ConD or ConD-OS at the same time point.

reduction of fetal blood glucose. In addition, after delivery, neonatal glucose supply is independent of the placenta. Together, these data demonstrate that the reduction in fetal and neonatal blood glucose concentrations in the HF-fed prepregnant mice may not be caused by reduced glucose supply from the mother or the placenta.

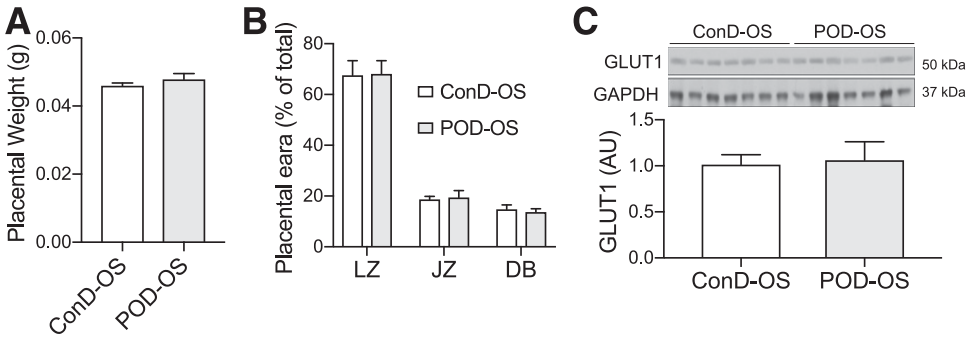

Figure 2—Prepregnant HF feeding did not alter the placental structure and GLUT1 expression. Placentas were collected at E18.5 through cesarean section. *A*: Tissue weight was determined by using an analytic balance (n = 46–58). *B*: Placental cross-section areas were determined using the images from hematoxylin and eosin–stained placental sections (n = 12). DB, decidua basalis; JZ, junction zone; LZ, labyrinth zone. *C*: Protein levels of GLUT1 and GAPDH were measured using Western blotting with a represented sample from each litter (n = 14). AU, arbitrary units.

Prepregnant HF Feeding Increased Phosphorylation of AKT in Fetal and Neonatal Liver and Skeletal Muscle

In adults, insulin stimulates glucose uptake in most metabolically active tissues and plays an essential role in controlling glucose metabolism. To study the effect of insulin-directed glucose disposal in fetal and neonatal mice, we injected insulin into normal C57BL/6 fetuses (E18.5, placenta-attached) and neonates (P1). As shown in Supplementary Fig. 2*A* and *B*, 10 min after insulin injection, blood glucose concentrations were significantly reduced in both fetuses and neonates, demonstrating significantly active insulin-stimulated glucose disposal. Insulin treatment also robustly increased phosphorylation of AKT in livers and skeletal muscles (Supplementary Fig. 2*C* and *D*). Therefore, like adults, insulin stimulated insulin signaling in metabolically active tissues and glucose disposal during the perinatal period.

We then compared the phosphorylation levels of AKT in skeletal muscles and livers of fetuses and neonates from PODs and ConDs at the fed state, which represents a stage of insulin stimulation compared with fasting. Phosphorylation levels of AKT were significantly increased in skeletal muscles and livers of fetuses and neonates from PODs (Fig. 3*A* and *B*). These results indicate that prolonged prepregnant HF feeding increased insulin signaling in metabolically active tissues in POD-OS.

Prepregnant HF Feeding Increased Fetal and Neonatal Pancreatic β-Cell Mass and Blood Insulin Concentrations

Elevated phosphorylation of AKT in fetal and neonatal tissues from PODs suggests that enhanced insulin signaling may be responsible for the decrease in their blood glucose concentrations. We therefore measured fetal and neonatal blood insulin concentrations (Fig. 4*A*). Similar to rats (23–25), fetal mouse blood insulin concentrations were remarkably higher than that of dams. After delivery, the neonatal blood insulin concentration rapidly declined (Fig. 4*A*). Despite the decrease in blood glucose levels of POD-OS, significantly higher levels of blood insulin were detected in fetuses and neonates from PODs (Fig. 4*A*). In parallel with increased blood insulin concentrations, the area of insulin-positive β-cell clusters from fetal and neonatal pancreases of POD-OS was significantly greater than those of ConD-OS (Fig. 4*B* and *C*). In addition, high glucose–induced but not low glucose–induced insulin secretion rates from isolated pancreatic islets of POD-OS were significantly higher than those from ConD-OS (Fig. 4*D* and *E*). These results indicate that increased β-cell mass and insulin secretion might contribute to prepregnant HF feeding–induced fetal and neonatal hyperinsulinemia.

Placental-Specific *Atgl* Gene Knockout Attenuated Prolonged Prepregnant HF Feeding–Induced Hyperinsulinemia and Decreased Perinatal Blood Glucose Concentrations

Maternal obesity increases fetal FA supply (21,26,27). Almost all maternal-supplied FAs (~97%) to the placenta are esterified into phospholipids and TGs in trophoblast cells (28–30). FA esterification and lipid droplet storage in trophoblast cells are considered as intermediate steps in the transfer of FAs to the fetus by the placenta (31). Similar to the effects of HF feeding during pregnancy and maternal obesity (21,27), prepregnant HF feeding significantly increased placental TG content (Supplementary Fig. 3*A* and *B*). As with adipocytes, lipolysis is essential to release FAs from TG. Consistent with human studies (32), significantly elevated protein levels of adipose TG lipase (ATGL), the key lipolytic enzyme, were observed in placentas of POD-OS (Fig. 5*A*). Therefore, by mating $Atgl^{f/f}$;$Tpbpa\text{-}Cre^{+/-}$ mice (Fig. 5*B*), we created littermates with the $Atgl^{f/f}$;$Tpbpa\text{-}Cre^{+/-}$ (pAtglko) or $Atgl^{f/f}$;$Tpbpa\text{-}Cre^{-/-}$ (Con) genotype (Fig. 5*C*) and studied the role of placental FA supply in prepregnant maternal HF feeding–induced hyperinsulinemia. As expected, remarkably increased placental TG contents were observed in the junctional zone of placentas from pAtglko fetuses (Fig. 5*D* and *E*). Significant reductions in fetal blood FFA concentrations and fetal body weight but not placental weight or blood TG concentrations were detected in pAtglko littermates (Fig.

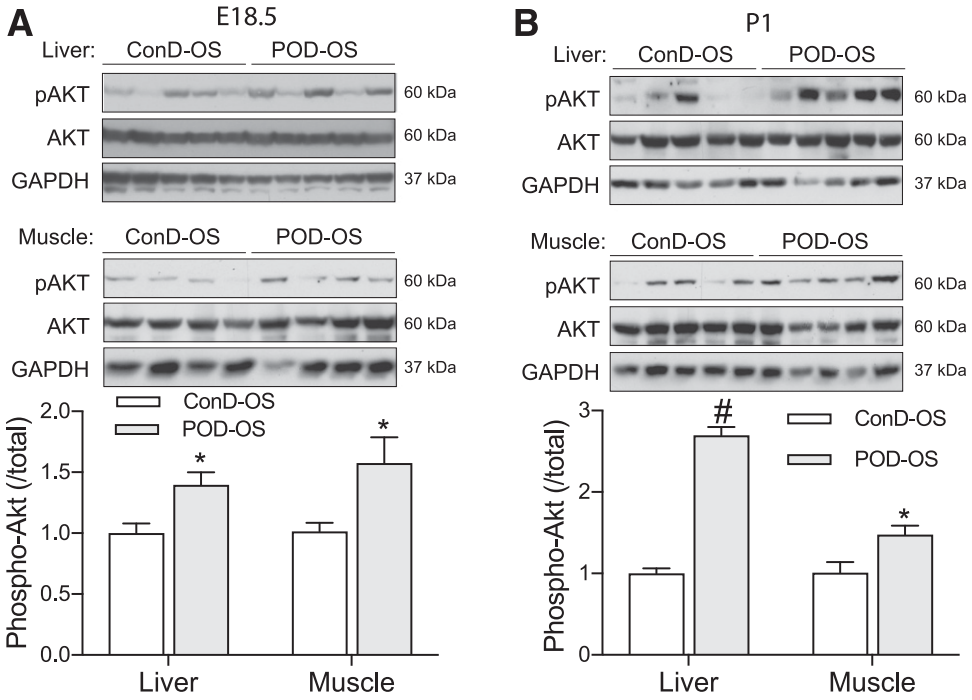

Figure 3—Prepregnant HF feeding increased insulin signaling in fetuses and neonates. Significantly increased phosphorylation of AKT (pAKT) protein levels were observed in POD-OS fetuses (*A*) and newborn pups (*B*) (n = 18). #$P < 0.01$, *$P < 0.05$ vs. ConD-OS.

5*F–I*). However, blood glucose concentrations of pAtglko fetuses and neonates were significantly increased (Fig. 5*J*). Elevated blood glucose concentrations in pAtglko fetuses would be caused by a decrease in glucose metabolism and/or an increase in placental glucose supply. However, slightly reduced placental mass (Fig. 5*H*) and similar expression levels of GLUT1 in the placentas (Fig. 5*K*) do not support any causal role of changes in placental glucose supply in producing the elevated pAtglko fetal blood glucose levels, although this conclusion would need to be measured directly to prove. Relatively decreased fetal glucose metabolism would competitively limit the transfer of glucose from the placenta in the pAtglko fetuses. In contrast to elevated blood glucose concentrations, a significant reduction in blood insulin concentration was observed in pAtglko fetuses and neonates (Fig. 5*L*). Significant reductions in the area of insulin-positive β-cells were also detected in pancreases from pAtglko fetuses and neonates (Fig. 5*M*). These results indicate that genetic blocking of lipolysis in the placenta induces perinatal hypoinsulinemia and secondary hyperglycemia in mice.

We then fed the mice the prepregnant HF diet and compared blood glucose and insulin concentrations between pAtglko and Con (*Atgl*$^{f/f}$;*Tpbpa-Cre*$^{-}$) littermates. As expected, prepregnant HF feeding induced an obese phenotype of dams (Supplementary Fig. 4*A*). HF feeding reduced fetal body weight without alteration in litter size (Supplementary Fig. 4*B* and *C*). Significantly increased blood insulin concentrations were observed in PODs (Supplementary Fig. 4*D*), while no remarkable changes in maternal blood glucose or FFA concentrations were observed (Supplementary Fig. 4*E* and *F*). Similar to the above results in C57BL/6 mice, maternal HF feeding significant reduced fetal blood glucose concentrations and increased blood insulin concentrations in Con littermates (Fig. 5*N* and *O*). However, for pAtglko littermates, the effects of prepregnant maternal HF feeding on fetal blood glucose and insulin levels were attenuated (Fig. 5*N* and *O*).

DISCUSSION

Maternal obesity is an important risk factor for neonatal hypoglycemia (1–7). Our current study revealed that prolonged prepregnant HF feeding of C57BL/6 mice creates a mouse model of pregravid obesity. Despite the difference between rodent and human pregnancy, this pregravid obese mouse model resembles maternal obesity–associated neonatal hypoglycemia in humans. Our study further demonstrated that prolonged maternal HF feeding impaired offspring glucose metabolism even before birth. Most importantly, our study revealed that placental FA supply plays an important role in fetal β-cell and islet development. Prepregnant HF feeding induces fetal and neonatal hyperinsulinemia by increasing fetal FA supply and β-cell mass.

As in adults, glucose is an essential nutrient for both fetuses and neonates, and hypoglycemia in both adults and neonates is a known risk factor for causing many adverse neurological consequences when severe, recurrent, and prolonged. Compelling data from human studies have

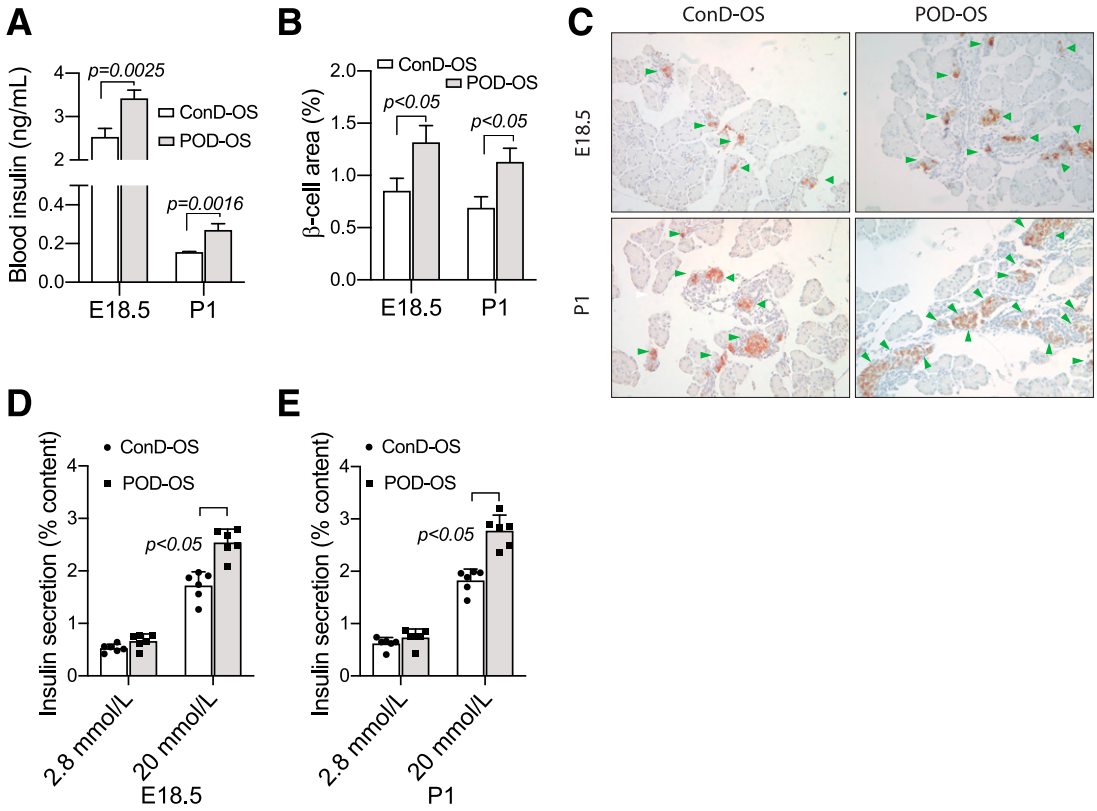

Figure 4—Prepregnant HF feeding increased fetal and neonatal blood insulin concentrations and pancreatic β-islet area. *A*: Serum insulin concentrations were determined by using an ELISA kit. Pancreases were collected at E18.5 or P1 using a dissection microscope. Pancreatic β-islet areas were probed by immunofluorescence (*B*) or immunohistochemistry (green arrowheads) (*C*) using an antibody against insulin. *B*: The relative β-islet area was calculated using immunofluorescent images. Glucose-stimulated insulin secretion was performed using handpicked β-islets (10 per well). Insulin concentrations in blood and medium or extraction were determined using an ELISA kit. $n = 24$ (*A*), $n = 12$ (*B* and *C*), and $n = 6$ (*D* and *E*).

demonstrated that maternal obesity plays a causal role in neonatal hypoglycemia (1–7). Using an HF feeding approach, the current study in mice reveals that prolonged prepregnant HF feeding significantly increases maternal adiposity and reduces blood glucose concentrations of newborn pups. To our knowledge, this is the first mouse study confirming that prepregnant HF feeding reduces neonatal blood glucose concentrations. Interestingly, our study also reveals that despite an increase of maternal adiposity, HF feeding during pregnancy did not reduce neonatal blood glucose concentrations. These results indicate that pregravid obesity but not excessive weight gain during pregnancy impairs neonatal glucose metabolism in mice. More studies are warranted to investigate the mechanisms underlying the different effects of pregravid obesity and EWG on offspring glucose metabolism. For example, compared with dams that received the HF diet only during pregnancy, the prepregnant HF-fed dams had a significantly longer duration of obesity and a greater level of adiposity during early pregnancy (Fig. 1*A*–*C*). This indicates that the duration of maternal obesity, especially obesity before and during in early pregnancy, may be important in reducing offspring blood glucose during the perinatal period.

Our study also showed that insulin injection robustly reduced blood glucose concentrations and increased the activation of insulin signaling in both placenta-attached fetuses and newborn mice. Insulin infusion also reduces fetal sheep blood glucose concentrations by increasing glucose utilization in fetal insulin-sensitive tissues (33,34). Therefore, insulin plays an important role in controlling glucose metabolism in fetuses and neonates. Because blood insulin and AKT phosphorylation levels were significantly increased in POD-OS, we propose that maternal obesity reduces fetal and neonatal blood glucose concentrations via increased insulin that promotes glucose utilization in insulin-sensitive tissues.

Insulin cannot pass through the placenta. Circulating fetal blood insulin is produced by fetal pancreatic β-cells. Despite the observation that β-cell and islet structures mature after term birth, insulin-expressing β-cell clusters can be identified in fetal mice by E15.5. In parallel with elevated blood insulin concentrations, significantly larger insulin-positive β-cell areas were observed in pancreases from POD-OS (Fig. 4*B* and *C*), indicating that prolonged HF feeding in prepregnant dams produces maternal obesity that increases β-cell mass. Similar observations have

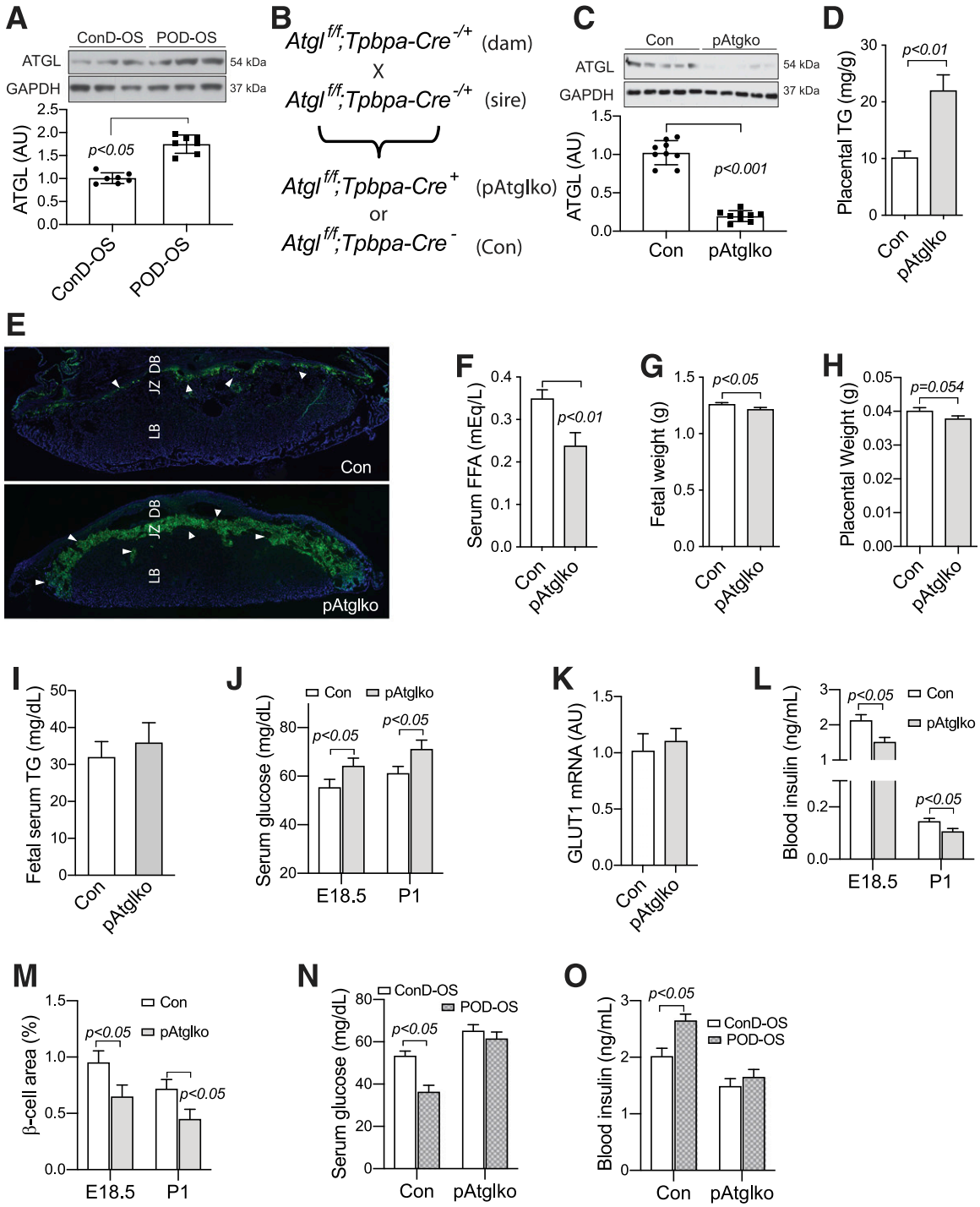

Figure 5—Placenta-specific *Atgl* gene knockout attenuated maternal HF feeding–induced reduction in fetal and neonatal blood glucose. *A*: The significantly elevated protein level of ATGL was observed in placentas of POD-OS ($n = 8$). *B*: The breeding scheme to produce pAtgIko and Con littermates. *C*: ATGL protein levels were robustly reduced in placentas from pAtgIko fetuses. *D* and *E*: A significant increase in placental TG content (arrowheads) was observed in placentas of pAtgIko ($n = 12$). Decreases in blood FFA (*F*), fetal weight (*G*) but not placental weight (*H*) and fetal blood TG (*I*) were detected in pAtgIko ($n = 44–62$). Remarkable changes in blood glucose concentrations (*J*), insulin concentrations (*L*), and β-islet areas (*M*) were found in pAtgIko fetuses and neonates. Some dams were fed the HF diet before pregnancy. *N* and *O*: The effects of prolonged prepregnant HF feeding on fetal glucose and insulin were attenuated in pAtgIko mice ($n = 46–57$). AU, arbitrary units.

been made in sheep, in which maternal obesity increases fetal blood insulin concentrations and β-cell mass (35). Interestingly, our study also found that glucose-stimulated insulin secretion rates were significantly greater in islets isolated from POD-OS (Fig. 4*D* and *E*). We therefore propose that increased β-cell mass increases blood insulin

concentrations in fetuses and newborns from prepregnant HF-fed dams. Increased glucose-stimulated insulin secretion may also contribute to higher in vivo insulin concentrations in the prepregnant HF progeny given our finding of greater in vitro glucose-stimulated insulin secretion in islets isolated from POD fetal mice. This remains to be tested by measuring in vivo glucose-stimulated insulin secretion in POD and control fetal mice.

Maternal obesity usually accompanies insulin resistance and gestational diabetes mellitus, suggesting that excessive fetal glucose supply induces hyperinsulinemia that causes neonatal hypoglycemia after abruptly stopping placental glucose supply at birth. One surprising observation of this study is that prepregnant maternal HF feeding reduces blood glucose concentrations not only in neonates but also in fetuses. Furthermore, the apparent reduction of transplacental glucose gradient and unaltered placental structure and GLUT1 protein levels of POD-OS do not support the concept that maternal obesity increases placental glucose supply in mice, at least by E18.5. Because the samples of this study were collected at late pregnancy and after birth and prepregnant obesity induces maternal insulin resistance, we cannot completely rule out the contribution of fetal glucose supply in early pregnancy to maternal obesity–associated neonatal hypoglycemia, although this would be less likely given that fetal β-cells and insulin secretion are not present until after day E15.5 and insulin secretion in response to glucose is not robust until close to term and after birth. Furthermore, maternal blood glucose concentrations of PODs at early pregnancy were comparable to ConDs (Supplementary Fig. 1*B*).

HF feeding increases β-cell mass in adult mice (36,37). Although many mechanisms underlie HF diet–induced β-cell expansion, dietary FA plays an important role in this process. Studies have demonstrated that maternal HF feeding and obesity increase fetal FA supply (21,26). However, the effects of FAs on fetal pancreatic β-cell development have not been studied (26). Unlike adults, fetal FA supply is controlled by the placenta. We took advantage of the nature of FA reesterification and lipolysis in trophoblast cells (28–30) and created a mouse model with placenta-specific *Atgl* gene knockout. Although lipolysis was primarily blocked in the junctional zone of the placenta in this mouse model, the significant reduction in fetal blood FA and body weights indicates that the blocked lipolysis reduced placental FA supply to the fetus. The remarkable decrease in blood insulin concentrations and the insulin-positive β-cell area in the pAtglko littermates confirms a key role of placental FA supply in fetal β-islet development. We realize that blocking lipolysis in the junction zone may alter the transport of other nutrients and/or placental hormonal expression, which could affect fetal β-cell development. However, the current study provides direct evidence indicating that placental lipolysis and FA supply are involved in fetal β-cell development. By using this genetic mouse model, our study also demonstrates that prolonged maternal HF feeding increases fetal and neonatal β-islet mass and blood insulin concentrations through increased placental FA supply. In adults, short-term HF feeding and FA treatment enhance β-cell proliferation and insulin secretion (36,38). However, chronic FA exposure causes β-cell apoptosis and reduction of insulin secretion, known as lipotoxicity (37,39,40). Therefore, we are pursuing separate projects to investigate how placental FA supply regulates fetal β-cell and islet development and the underlying mechanisms through which maternal obesity increases fetal β-cell mass.

Consistent with other rodent studies (18–20), we found that HF feeding both before and during pregnancy significantly inhibited fetal growth. This is despite higher fetal blood insulin concentrations in the POD-OS. The Pedersen hypothesis proposes that increased fetal insulin concentrations as a result of increased glucose transfer across the placenta increases fetal growth (8). Therefore, our data appear to contradict aspects of this hypothesis, specifically, that higher insulin concentrations were not associated with higher fetal weight in the POD-OS. There are several possible reasons for this discrepancy. The Pedersen hypothesis specifically relates to increased glucose transfer to the fetus, which we did not find evidence for in our model. Second, fetal growth is regulated by various factors, including placental nutrient supply and several hormones (8,41,42). We speculate that maternal HF feeding may impact fetal hormonal pathways that counteract the stimulatory effect of hyperinsulinemia on fetal growth in this model. The observations of lower fetal weight in both experimental groups but hyperinsulinemia only in the POD-OS fetuses (Fig. 1*D* and *H*) support this assertion. Finally, species difference might underlie the variance with the Pedersen hypothesis.

In summary, the current study demonstrates that prolonged prepregnant HF feeding of C57BL/6 mice resembles the major metabolic characteristics of maternal obesity–induced neonatal hypoglycemia. Interestingly, prolonged prepregnant HF feeding reduces blood glucose concentration not only in neonates but also in fetuses during late pregnancy. This study also reveals that placental FA supply plays an important role in β-cell and islet development and that elevated FA supply is responsible for maternal obesity–induced fetal and neonatal hyperinsulinemia. Reduction in fetal and neonatal blood glucose concentration is a consequence of maternal obesity–enhanced fetal β-cell and islet development.

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