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The Essential Role of Pancreatic α-Cells in Maternal Metabolic Adaptation to Pregnancy

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Pancreatic α-cells are important in maintaining metabolic homeostasis, but their role in regulating maternal metabolic adaptations to pregnancy has not been studied. The objective of this study was to determine whether pancreatic α-cells respond to pregnancy and their contribution to maternal metabolic adaptation. With use of C57BL/6 mice, the findings of our study showed that pregnancy induced a significant increase of α-cell mass by promoting α-cell proliferation that was associated with a transitory increase of maternal serum glucagon concentration in early pregnancy. Maternal pancreatic GLP-1 content also was significantly increased during pregnancy. Using the inducible Cre/loxp technique, we ablated the α-cells (*α-null*) before and during pregnancy while maintaining enteroendocrine L-cells and serum GLP-1 in the normal range. In contrast to an improved glucose tolerance test (GTT) before pregnancy, significantly impaired GTT and remarkably higher serum glucose concentrations in the fed state were observed in *α-null* dams. Glucagon receptor antagonism treatment, however, did not affect measures of maternal glucose metabolism, indicating a dispensable role of glucagon receptor signaling in maternal glucose homeostasis. However, the GLP-1 receptor agonist improved insulin production and glucose metabolism of *α-null* dams. Furthermore, GLP-1 receptor antagonist Exendin (9-39) attenuated pregnancy-enhanced insulin secretion and GLP-1 restored glucose-induced insulin secretion of cultured islets from *α-null* dams. Together, these results demonstrate that α-cells play an essential role in controlling maternal metabolic adaptation to pregnancy by enhancing insulin secretion.

Pregnancy is a relatively short physiological process during a woman's life. To accommodate embryo implantation, placental and fetal development, and prepare for delivery and lactation, maternal metabolism goes through a series of adaptations during this short period. Although many systems are involved, available data demonstrate that insulin plays a dominant role in regulating these metabolic adaptations. There is a significant expansion of maternal pancreatic islet mass and insulin production to meet the increased demand for insulin. Interestingly, despite reduced insulin sensitivity in maternal metabolically active tissues and increased hepatic glucose production (1,2), maternal blood glucose concentration gradually decreases due to the increasing consumption of glucose by the placenta and fetus as gestation proceeds (3–6). Therefore, the progressive elevation of maternal blood insulin concentrations and a reduction in blood glucose concentrations are hallmarks of maternal metabolic adaptations to pregnancy (7–10).

α-Cells are the second most common primary pancreatic endocrine cells and make up 35–40% of human islet cellular content (11–13). In response to fasting and decreased blood glucose concentration, α-cells secrete glucagon to promote hepatic glucose production (14,15). Glucagon also enhances insulin secretion via intraislet paracrine effects (16,17). Recent studies have shown that the α-cells also secrete glucagon-like peptide 1 (GLP-1) (18–22), which promotes glucose-induced insulin secretion (23). Although both glucagon receptor (GCGR) and GLP-1 receptor (GLP-1R) are expressed in pancreatic β-cells, intraislet glucagon and GLP-1 enhance insulin secretion mainly through GLP-1R (17,24,25).

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Given the importance of α-cells in controlling metabolism, the role of α-cells in maternal metabolic adaptation during pregnancy has not been systemically investigated. Previous human and rat studies have reported that pregnancy increases maternal blood glucagon concentrations at some time points (26–29). Although *Gcgr* gene deletion reduced maternal blood glucose concentrations (30,31), the confounding effects of whole-body gene knockout on maternal islet development in early life cannot be ruled out. Conflicting data also were reported regarding adaptative changes in the maternal α-cell population and glucagon production during pregnancy (32–34). Therefore, for the studies reported in this article, we used mouse models to measure the responses of α-cells to pregnancy and their regulatory effects on maternal metabolism. Our results showed that, in parallel with β-cell mass expansion, there also was a significant increase in the α-cell population during pregnancy. Unlike the transitory increase in maternal serum glucagon concentrations in early pregnancy, pancreatic GLP-1 and glucagon contents were robustly increased during most of gestation. α-Cell ablation improved glucose tolerance in nonpregnant mice but robustly increased maternal serum glucose concentrations during pregnancy. Importantly, α-cell ablation significantly impaired pregnancy-induced insulin production, and GLP-1R agonist injection restored serum insulin concentrations in *α-null* dams. GLP-1R antagonist Exendin (9-39) (Ex9) significantly attenuated pregnancy-enhanced insulin secretion. Together, these results reveal a vital role of pancreatic α-cells in maternal insulin production.

RESEARCH DESIGN AND METHODS

Materials

The anti-glucagon antibody was from R&D Systems (Minneapolis, MN). Antibodies against insulin and Ki67 were from Abcam (Cambridge, MA). The total GLP-1 ELISA kit, TRIzole, SuperScript III reverse transcriptase, and Oligo(dT)$_{12\text{-}18}$ primer and the Alexa Fluor–conjugated goat anti-mouse, rabbit, and sheep antibodies were from Invitrogen (Carlsbad, CA). Glucose, glucose oxidase, glucagon, GLP-1 (7-36) amide, Ex9, tamoxifen (Tmx), BSA, Ficol, DMEM, and RPMI medium were from Sigma-Aldrich (St. Louis, MO). The mouse insulin and glucagon ELISA kits were from Mercodia (Uppsala, Sweden). The in situ cell death detection kit was from Roche (Basel, Switzerland). Anti–glucagon receptor antibody REMD25.9 was provided by REMD Biotherapeutics Inc. (Camarillo, CA). Semaglutide (Sem) was obtained from Novo Nordisk (Plainsboro, NJ).

Experimental Animals

ROSA26-$^{eGFP\text{-}DTA}$ mice (named as *DTA*) (35,36), *Gcg*^{CreErt2} mice (37), *Gcg*^{iCre} (38), *Ai9* (39), *mTmG* (40), and C57BL/6 mice were from The Jackson Laboratory (Bar Harbor, ME). All of these transgenic mice have the C57BL/6J genetic background. *DTA*, *mTmG*, or Ai9 mice were crossed with *Gcg*^{CreErt2} or *Gcg*^{iCre} mice to create *DTA;Gcg*^{CreErt2}, *mTmG;Gcg*^{CreErt2}, *Ai9;Gcg*^{CreErt2}, or *Ai9;Gcg*^{iCre} mice. To ablate α-cells and allow intestinal L-cell recovery, 8-week old *DTA;Gcg*^{CreErt2} (named as *α-null*) mice were orally gavaged with Tmx (1 mg daily in 100 μL corn oil) for two consecutive days. Since our preliminary data showed that 2-day Tmx treatment ablates ∼96% of α-cells in *DTA;Gcg*^{CreErt2} mice, we selected a 2-day gavage to minimize potential side effects of Tmx on pregnancy. Mating and studies were performed 1 month after Tmx treatment to allow L-cell recovery. Ten- to twelve-week-old nulliparous female mice were randomly selected for mating. Pregnancy was determined by the presence of a vaginal plug and was assigned the embryonic day (E)0.5. Glucose tolerance tests (GTT) were performed after overnight fasting with intraperitoneal glucose injection (2 g/kg). Serum samples were prepared, and glucose concentration was determined with use of glucose oxidase. Some *α-null* dams received glucagon (50 μg/kg at 1 min before glucose injection) or GLP-1R agonist Sem (6 μg/kg s.c. 10 min before glucose injection) during intraperitoneal GTT (21,41). Maternal tissues, placentas, and fetuses were collected in the fed state. Pancreatic glucagon and GLP-1 were extracted via homogenizing tissue in 70% ethanol and 20 mmol/L HCl.

For tracking of α-cell neogenesis, nonpregnant *Ai9;Gcg*^{CreErt2} female mice were orally gavaged with Tmx (2 mg daily in 100 μL corn oil) for five consecutive days, which resulted in the labeling of nearly all α-cells. Two weeks later, they were mated with C57BL/6 sires. Pancreases were collected at E18.5, and immunofluorescence (IF) was performed with an anti-glucagon antibody. To detect transdifferentiation between α-cells and β-cells during pregnancy, we used the *Ai9;Gcg*^{iCre} mice in which all GCG-expressing cells were labeled with a red fluorescent protein (RFP). Pancreases were collected at indicated gestational ages. Glucagon and insulin coexpressed cells were detected by Gcg-directed RFP and IF with anti-insulin antibodies. To check GCG promoter–directed Cre expression, we gavaged *mTmG;Gcg*^{CreErt2} mice with Tmx for five consecutive days.

Experiments using mouse models were carried out according to the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) guidelines with approval from the University of California San Diego Animal Care and Use Committee.

Immunohistochemistry, IF, and β-Islet Morphometric Analysis

Tissues were fixed in 4% paraformaldehyde or 10% neutral-buffered formalin and then processed and embedded in optimal cutting temperature compound or paraffin. For immunohistochemistry, tissue sections were blocked with 2% H_2O_2 in PBS and then heated in 0.1 mol/L pH 6.0 citrate buffer for 15 min at 95°C for antigen retrieval. After second-round blocking, immunostaining of insulin was done using an anti-insulin primary antibody (10 μg/mL)

or rabbit serum (for a negative control) for 4 h. The sections were visualized with 3,3′-diaminobenzidine (Vector Laboratories, Burlingame, CA) at room temperature for 1.5 min and counterstained with hematoxylin. α-Cells, β-cells, and pancreas areas were measured with immunohistochemistry (IHC)-stained series of sections with the microscope software (BZ-X800E; Keyence, Laguna Hills, CA). α-Cell and β-cell masses were measured with the use of glucagon- or insulin-stained series of sections. We determined the percentage of α-cell or β-cell area in the pancreas by dividing the area of glucagon- or insulin-positive cells in one section by the pancreas area. The α-cell or β-cell mass was calculated by multiplication of the pancreas weight by the percentage of α-cell or β-cell area as we previously described (4,42).

For IF, the slides were incubated in 1% SDS in PBS for 5 min to induce antigen retrieval. After blocking with BSA in PBS for 2 h, sections were incubated with anti-insulin, anti-glucagon, or anti-Ki67 antibody overnight at 4°C. After rinsing, the secondary antibody conjugated with Alexa Fluor 488 or 568 was applied to the slides and incubated for 2 h at room temperature. After washing, sections were mounted in DAPI Fluoromount-G (SouthernBiotech, Birmingham, AL) and visualized by fluorescent optical microscopy. The ratios of Ki67 and glucagon or insulin protein–positive cells were calculated with use of IF images. For each dam, 8–12 islets were randomly counted for Ki67-, glucagon-, or insulin-positive cells from different sections. The average percentage of Ki67-positive α-cells or β-cells from each dam was used for statistical analysis. The apoptotic cells were detected with TUNEL staining. The tissue sections from *mTmG;Gcg*^{CreErt2} reporter mice were mounted in DAPI Fluoromount-G and visualized with fluorescent optical microscopy.

Pancreatic Islet Isolation and Glucose-Stimulated Insulin Secretion

The pancreatic islets were isolated by collagenase digestion and differential centrifugation through Ficol gradients as we previously described (4). For mRNA extraction, all islets were immediately homogenized in TRIzole. For glucose-stimulated insulin secretion (GSIS), size-matched islets were handpicked and incubated overnight in RPMI with 5 mmol/L glucose. One hour before the assay, the culture medium was changed to the Krebs-Ringer medium with 2 mmol/L glucose. The islets were then stimulated with the addition of glucose (20 mmol/L) for 1 h with or without 1 μmol/L Ex9 or 10 μg/mL REMD25.9. The medium was collected at the end of stimulation. The insulin left in the islets was extracted and measured with an ELISA kit (4,43).

Statistical Analysis

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

Data and Resource Availability

The data sets and reagents generated during the current study are available from the corresponding author on reasonable request.

RESULTS

Pregnancy Increased α-Cell Mass and Intraislet GLP-1 Production

Pregnancy significantly increases β-cell mass in rodents through enhancing β-cell proliferation (44,45). Similarly, our measurement revealed a significant increase of glucagon-positive tissue masses of pregnant mice from E6.5 to E18.5 (Fig. 1*A* and *B*). The cellular sizes of glucagon-positive cells were the same between basal and E15.5 (Supplementary Fig. 1*A*). There was no significant change in the ratios of glucagon- and insulin-positive area within the islet (Fig. 1*C*), indicating a proportional increase of α- and β-cells during pregnancy. The proliferation marker Ki67 protein–positive rates of α-cells were significantly increased during early pregnancy and midpregnancy (Fig. 1*D*). We also labeled α-cells with tdTomato (RFP) before pregnancy using *Ai9*;*Gcg*^{CreErt2} mice and then studied any precursor-directed α-cell neogenesis during pregnancy. No significant increase of RFP-negative and glucagon-positive cells was detected during pregnancy, indicating no α-cell neogenesis during pregnancy (Supplementary Fig. 1*B*). We also studied the transdifferentiation between α- and β-cells during pregnancy using *Ai9*;*Gcg*^{iCre} mice, in which all α-cells were labeled with RFP. There was no significant difference in insulin and RFP coexpressed cells between basal and E15.5 (mean ± SEM 0.14 ± 0.07% of α-cells vs. 0.18 ± 0.11% of α-cells [Supplementary Fig. 1*C*]). Consistent with a recent report (33), our data indicate that the increase of the α-cell number and tissue mass during pregnancy is not due to increased transdifferentiation from β-cells. Almost no TUNEL-positive α-cells were detected (data not shown), indicating no α-cell apoptosis during pregnancy. Together, these results indicate that pregnancy increases pancreatic α-cell mass by enhancing α-cell proliferation.

Despite a noticeable increase of α-cell mass during pregnancy, a significant increase of serum glucagon concentrations was only detected at E6.5, and pancreatic glucagon contents were increased between E6.5 and E12.5 (Fig. 1*E* and *F*). However, pancreatic total GLP-1 contents were significantly increased at nearly all pregnancy time points (Fig. 1*G*).

α-Cell Ablation Induced Hyperglycemia and Hypoinsulinemia in Pregnant Mice

To study the role of α-cells in maternal metabolic adaptation, we created transgenic mice with Tmx-inducible α-cell

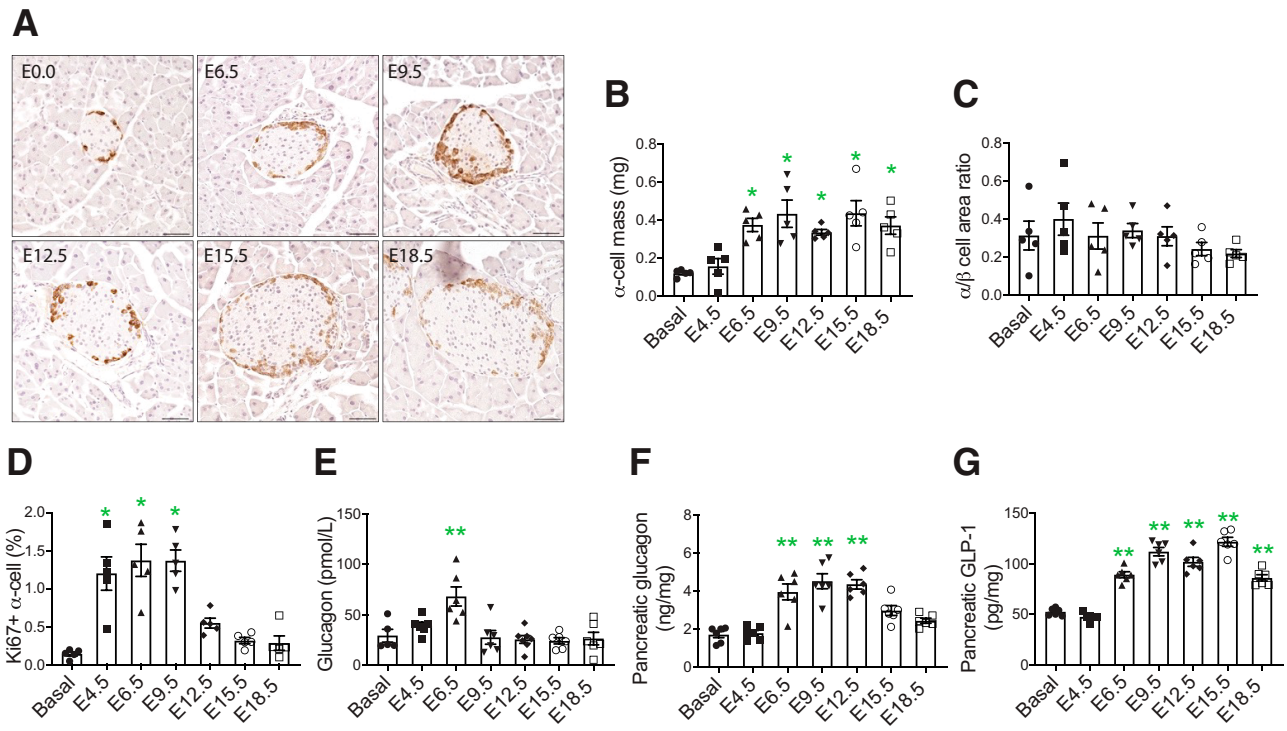

Figure 1—Pregnancy increases the α-cell mass and pancreatic GLP-1. Maternal tissue samples were collected from pregnant C57BL/6J mice at indicated embryonic ages. *A* and *B*: α-Cell mass was measured with use of IHC images of a series of pancreatic sections (dark brown in *A*). *C*: β-Cell and α-cell areas were determined with use of anti-insulin and anti-glucagon antibodies–stained IF images; then, the area ratio was calculated. *D*: Ki67-positive rates were calculated with use of anti-Ki67 and anti-glucagon antibodies–probed IF images. Glucagon (*E* and *F*) and total GLP-1 (*G*) levels were measured with use of fed serum (*E*) or acid-ethanol–extracted pancreatic tissues (*F* and *G*). *$P < 0.05$, **$P < 0.001$ vs. basal. The scale bar is 50 μm.

ablation (*α-null*) by crossing the *ROSA26-*$^{eGFP\text{-}DTA}$ mice (35,36) with preproglucagon promoter–directed Cre transgenic (*Gcg*^{CreErt2}) mice (37). Results showed that 2-day Tmx treatment depleted ∼96% of α-cells (Fig. 2*A*). A significant number of intestinal L-cells were also ablated (Fig. 2*A*) (Tmx vs. oil). Although preproglucagon is expressed in the brain (46), the *Gcg*^{CreErt2} did not direct any DNA recombination in PPG neurons (Supplementary Fig. 2*A*), which is consistent with others' reports (25,37). In addition, a recent study demonstrated that PPG neuron ablation exhibited no effect on insulin production and glucose metabolism (47).

Consistent with findings of previous studies (21,38, 48,49), ablated α-cells were not replenished in adult mice but L-cells were quickly regenerated (Fig. 2*A*). Serum GLP-1 concentrations were also recovered to control levels (Supplementary Fig. 2*B*). To focus on the effect of pancreatic α-cells, we used the mice 1 month after Tmx treatment. The same strategy has been used to let L-cells recover (21,25,49). Therefore, after L-cell replenishment, the *α-null* mice provided a unique model for study of the role of α-cells in maternal metabolic adaptation.

Like other α-cell–ablated mice (21,48,49), nonpregnant female and male *α-null* mice exhibited no significant changes in random-fed serum glucose concentrations, triglyceride (TG) concentrations (Fig. 2*B* and *C*), and fasting serum glucose concentrations (Fig. 2*D* and *E* [time 0]). However, an improved GTT was observed in male and female *α-null* mice (Fig. 2*D* and *E*). A similar phenotype of improved GTT was previously reported in mice with glucagon deficiency (50–53).

During pregnancy, significantly increased serum glucose and TG concentrations were observed in pregnant *α-null* mice (Fig. 2*F* and *G*). In contrast, serum insulin concentrations of *α-null* dams were significantly lower than those of the pregnant control (Con) dams (Fig. 2*H*). Of note, despite insulin resistance, Con dams' serum glucose concentrations were significantly decreased in late pregnancy (Fig. 2*F*), which is in line with findings of human and rodent studies (4–6). During GTT, serum glucose concentrations of *α-null* dams were significantly higher than in Con dams (Fig. 2*I*). Accordingly, lower serum insulin concentrations were detected in *α-null* dams (Fig. 2*J*). Together, these results show that α-cell ablation impairs maternal glucose metabolism due to reducing serum insulin concentrations.

α-Cell Ablation Showed No Significant Effect on β-Cell Proliferation but Significantly Decreased GSIS During Pregnancy

Increasing β-cell mass and insulin secretion are the main mechanisms underlying the progressive increase of

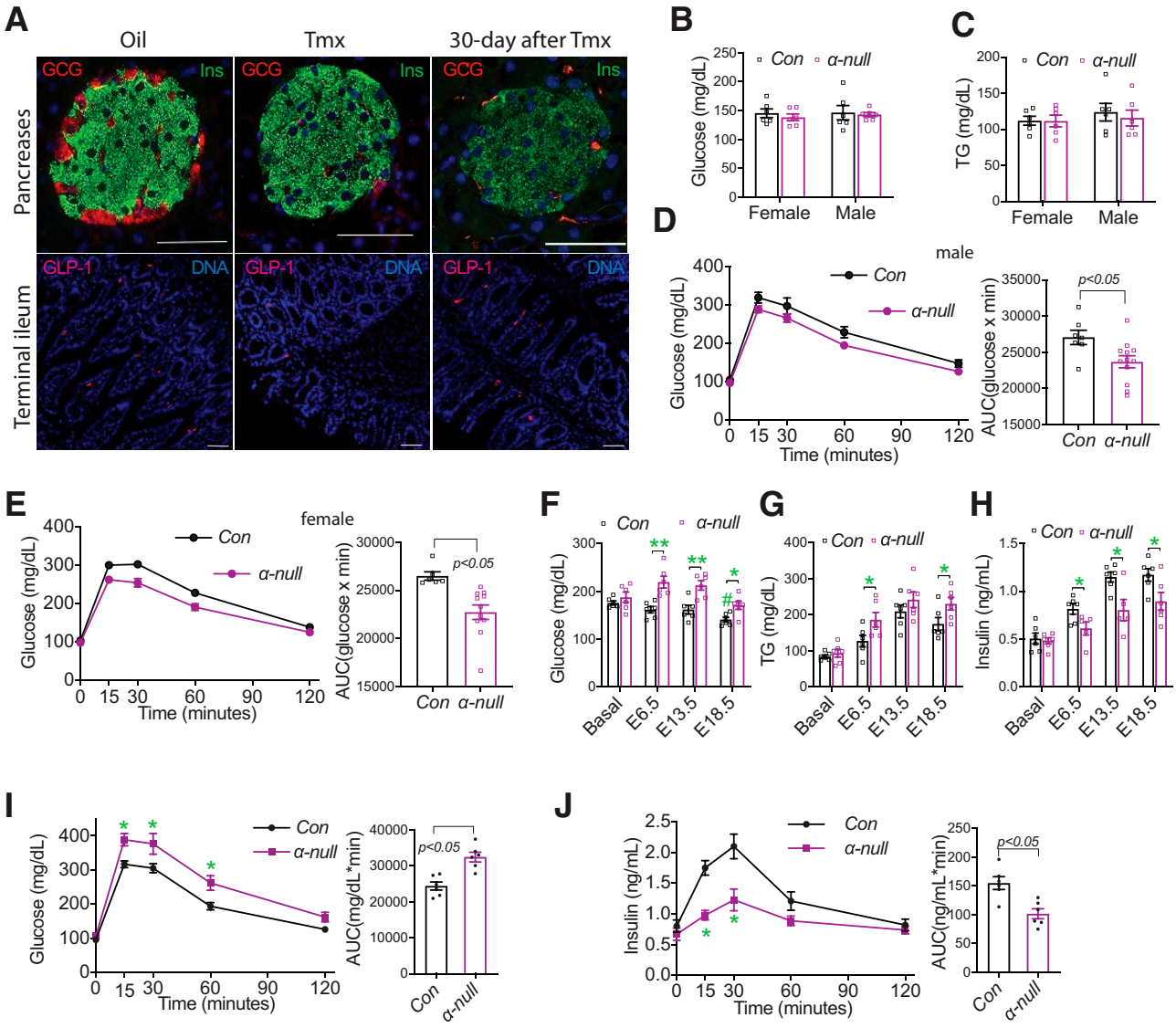

Figure 2—α-Cell ablation impaired maternal glucose metabolism and insulin production. Female *DTA*$^{+/+}$;*Gcg*$^{CreErt2+/-}$ mice were orally gavaged with Tmx (1 mg in 100 μL corn oil) or oil for two consecutive days. *A*: Tissues were collected 1 or 30 days after Tmx treatment. The α- and β-cells in the pancreases (top) and L-cells in the intestine (bottom) were probed via IF with anti-glucagon (GCG), insulin (Ins), or GLP-1 antibody. The scale bar is 50 μm. *B–E*: 1 month after Tmx treatment, glucose (*B*) and TG (*C*) concentrations were measured with use of serum samples in the fed state. Significantly improved intraperitoneal GTT was observed in both male (*D*) and nonpregnant female (*E*) *α-null* mice. *F–J*: Pregnancies were established 1 month after Tmx treatment. Random-fed serum samples were collected (~9:30–10:00 A.M.) at indicated gestational age, and serum glucose (*F*) and TG (*G*) concentrations were determined. *I* and *J*: Intraperitoneal GTT was performed at E11.5 after overnight fasting (n = 6–8). *$P < 0.05$, **$P < 0.001$ vs. Con, #$P < 0.05$ vs. basal. AUC, area under the curve.

maternal serum insulin concentrations during pregnancy. Our results showed that β-cell mass was comparable between Con and *α-null* mice before and during pregnancy (Fig. 3*A*). Similar percentages of Ki67-positive β-cells were also observed in Con and *α-null* dams (Fig. 3*B*), indicating that α-cells are dispensable in pregnancy-induced β-cell mass expansion.

We then performed a GSIS study using islets from age-matched nonpregnant control (*np-Con*), Con, and *α-null* dams. Basal insulin secretion rates were comparable between *np-Con,* Con, and *α-null* islets (Fig. 3*C*). As expected, glucose-induced insulin secretion rates were significantly higher in islets from Con dams compared with *np-Con* islets (Fig. 3*C*). However, glucose-induced insulin secretion rates were significantly attenuated in islets from *α-null* dams (Fig. 3*C*). These results indicate that α-cell ablation impaired glucose-enhanced insulin production during pregnancy.

Glucagon Receptor Antagonism Did Not Alter Maternal Glucose Metabolism

Glucagon regulates glucose metabolism mainly through the endocrine effects on the liver and other peripheral

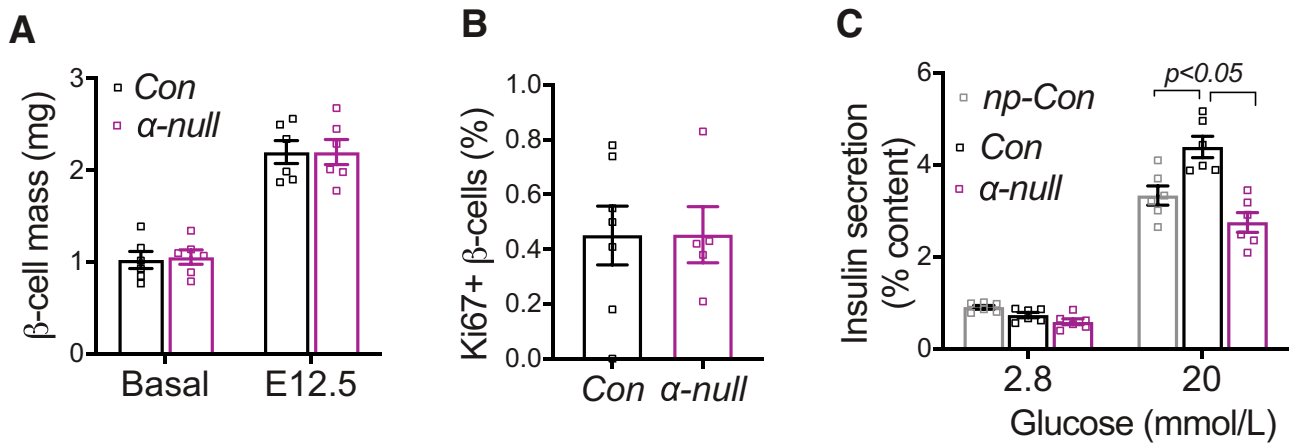

Figure 3—α-Cell ablation reduced glucose-induced insulin secretion in pregnant mice. The *α-null* mice were allowed 1-month recovery after Tmx gavage. Tissue samples were collected before pregnancy (basal) and at E12.5. Pancreatic sections were stained via IHC with anti-insulin antibody (*A*) and IF with anti-Ki67 and anti-insulin antibodies (*B*). *A*: β-Cell mass was determined with use of a series of section and IHC images. *B*: The averaged Ki67-positive β-cell rate from each dam was used for statistical comparison. *C*: Islets were prepared through collagenase perfusion and cultured overnight from *np-Con* and E12.5 dams. Size-matched islets were used for insulin secretion assay. The release rates of insulin into the medium were calculated.

tissues. The *α-null* dams had remarkably reduced serum glucagon concentrations and pancreatic glucagon and GLP-1 contents (Fig. 4*A–C*). However, pancreatic insulin contents were not altered in *α-null* dams (Fig. 4*D*). Glucagon receptor (*Gcgr*) gene knockout dams exhibited persistent hypoglycemia (30,31), which is opposite to the metabolic phenotypes of *α-null* dams. Therefore, we used glucagon receptor antibody REMD25.9 (REMD Biotherapeutics Inc.) to block GCGR signaling during pregnancy and further assess the endocrine effect of glucagon on maternal metabolic adaptation (54–56). Antibody injection avoids the developmental impact of systemic *Gcgr* gene knockout (30,31).

Blocking GCGR signaling increases α-cell mass and glucagon and pancreatic GLP-1 production (57–59). Significantly increased serum and pancreatic glucagon and GLP-1 contents and enlarged α-cell mass were observed in REMD25.9-treated dams (Fig. 4*E–H*), indicating a successful blockage of GCGR signaling. To our surprise, there was no significant alteration in random feeding serum glucose and insulin concentrations in REMD25.9-treated dams (Fig. 4*I* and *J*). Similarly, comparable fasting serum glucose and insulin concentrations were observed in REMD25.9-treated and Con dams (Fig. 4*K* and *L*). The metabolic phenotypes of *α-null* dams are strikingly different from those of REMD25.9-treated dams. The main difference between these two models is the opposite changes in pancreatic glucagon and GLP-1 contents (Fig. 4*A–G*). Since GLP-1R plays a dominant role in mediating the insulinotropic effect of both glucagon and GLP-1 (17,24,25), we performed intraperitoneal GTT of Con and REMD25.9-treated dams with or without GLP-1R antagonist Ex9. Ex9 injection significantly increased serum glucose concentrations, while it reduced GSIS, in both REMD25.9-treated and Con dams (Fig. 4*M* and *N*). Since GLP-1R mediates the regulatory effects of both glucagon and GLP-1, these results led us to propose that increased intraislet GLP-1 and glucagon might maintain insulin production and glucose metabolism in REMD25.9-treated dams. These data also support a previous study that demonstrated the importance of GLP-1R in maternal β-cell adaptation to pregnancy (32).

GLP-1R Agonist Increased Maternal Serum Insulin in *α-null* Dams, While GLP-1R Antagonist Ex9 Attenuated Pregnancy-Enhanced Insulin Secretion

The above results showed that α-cell ablation significantly reduced pancreatic glucagon and GLP-1 contents and insulin production during pregnancy. The α-cells promote insulin secretion through glucagon and GLP-1 (16,24,25). We injected glucagon or GLP-1R agonist Sem into *α-null* dams during GTT to determine which hormone mediates the regulatory effects. Results showed that Sem injection significantly improved serum glucose and insulin concentrations in *α-null* dams (Fig. 5*A* and *B*). However, glucagon injection did not improve serum glucose and insulin concentrations in *α-null* dams (Fig. 5*A* and *B*). Importantly, serum glucose concentrations during intraperitoneal GTT of Sem-treated *α-null* dams were even significantly lower than those of Sem-treated Con dams (Fig. 5*A*). Since serum insulin concentrations of Sem-treated *α-null* dams were restored to Sem-treated Con dam levels (Fig. 5*B*), we reasoned that decreased glucagon of *α-null* dams contributed to the significantly improved GTT of *α-null*–Sem dams. The increased glucose concentrations during GTT of GCG-treated *α-null* dams (Fig. 5*A*) further support the essential role of glucagon in maintaining serum glucose concentrations after fasting.

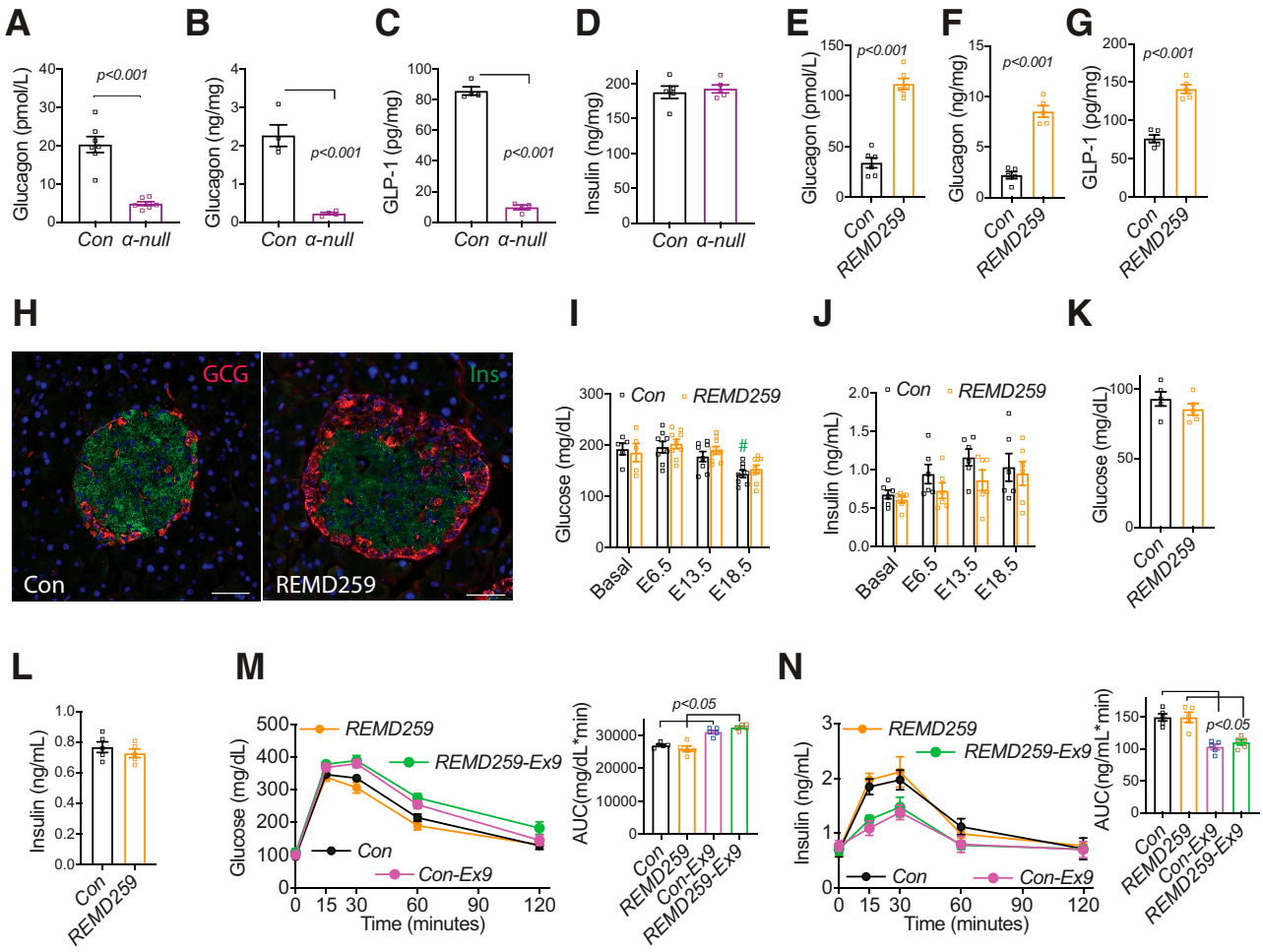

Figure 4—Glucagon receptor antagonism did not alter maternal glucose metabolism. *A–D*: Serum and pancreases were collected from Con and α-*null* dams at E18.5. Pancreatic hormones were extracted by acid-ethanol buffer. Serum glucagon (*A*) and pancreatic glucagon (*B*), total GLP-1 (*C*), and insulin (*D*) contents were determined with ELISA kits. *E–N*: Pregnant C57BL/6 mice received a weekly injection of glucagon receptor antibody REMD25.9 (7 mg/kg i.p.) or saline. REMD25.9 injection significantly increased serum glucagon (*E*) and pancreatic glucagon (*F*) and GLP-1 contents (*G*) (E18.5). Enlarged α-cell mass (*H*) was also observed in REMD-treated dams (E18.5). No noticeable differences in maternal feeding (*I* and *J*) and overnight fasting (*K* and *L*) (E13.5) serum glucose (*I* and *K*) or insulin concentrations (*J* and *L*) were detected during pregnancy. *M* and *N*: Intraperitoneal GTT was performed at E12.5 with injection of Ex9 (20 μg i.p. 15 min before glucose injection) or saline. $n = 5–6$. #$P < 0.05$ vs. basal. The scale bar is 50 μm. AUC, area under the curve.

We then repeated the GSIS of islets from α-*null* dams in the presence of GLP-1 and glucagon. Results showed that GLP-1 treatment restored GSIS rates of islets from α-*null* dams to the levels of islets from Con dams (Fig. 5*C*). Given the recovery of L-cells and normal serum GLP-1 in α-*null* mice (Fig. 2*A* and Supplementary Fig. 2*B*), these results indicate that the reduction of intraislet GLP-1 is most likely responsible for the decrease of insulin production in pregnant α-*null* mice.

Both GCGR and GLP-1R are expressed in β-cells and mediate the insulinotropic effects of glucagon and GLP-1 (17,24,25). For further verification of the pathway(s) through which α-cells regulate the adaptation of maternal β-cells to pregnancy, a GSIS was performed in the presence of GLP-1R or GCGR antagonist Ex9 or REMD25.9. Results showed no effect of Ex9 and REMD25.9 treatment on basal insulin secretion rates in all islets (Fig. 5*D*). Under high glucose conditions, insulin secretion rates were significantly reduced by Ex9 treatment in both islets from virgin and islets from pregnant mice (Fig. 5*D*). In contrast, the REMD25.9 treatment did not alter glucose-induced insulin secretion rates in either group. These results indicate that similar to GLP-1R in nonpregnant conditions (17,24,25), GLP-1R plays an essential role in glucose-induced insulin secretion during pregnancy.

DISCUSSION

Increased insulin production and circulating insulin concentrations are essential in regulating normal maternal metabolic adaptations to pregnancy (7–10). In addition to peripheral insulin resistance, inadequate insulin production

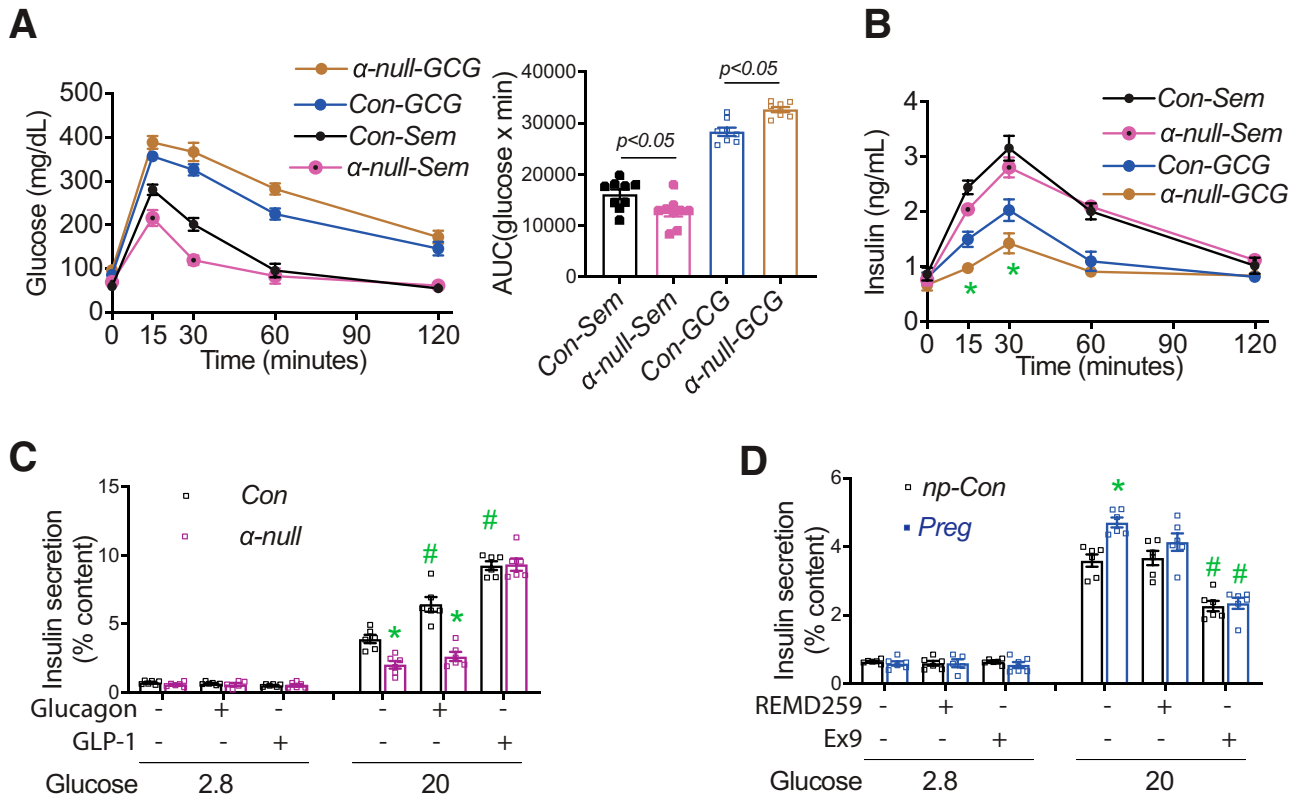

Figure 5—GLP-1R agonist restored glucose tolerance in *α-null* dams. *A* and *B*: Pregnancy of *α-null* or Con mouse was induced 1 month after Tmx treatment. Intraperitoneal GTT was performed with injection of glucagon (GCG) (50 μg/kg at 1 min before glucose injection) or GLP-1R agonist Sem (6 μg/kg s.c. 10 min before glucose injection) on E12.5. *C*: Islets were prepared from *α-null* and Con dams at E12.5 and cultured overnight. GSIS was performed with the presence of glucagon (10 nmol/L) and GLP-1 (7-36) amide (0.3 nmol/L) in indicated wells. *D*: GSIS was performed using size-matched islets from *np-Con* and pregnant (Preg) C57BL/6 mice (E12.5) with the presence of REMD15.9 (10 μg/mL) and Ex9 (1 μmol/L).

during pregnancy leads to hyperglycemia and gestational diabetes mellitus (9,10,60). The current study reveals an essential role of pancreatic α-cells in pregnancy-enhanced insulin production.

β-Cells and α-cells are primary endocrine cells in pancreatic islets. However, the role of α-cells in islet adaptation to pregnancy has not been specifically studied. Our data show that, in parallel with the expansion of β-cell mass, α-cell mass also significantly increases during pregnancy. Others have reported similar phenotypes in studies in pregnant mice (34,61). Our results also indicate that significantly increased proliferation in early pregnancy most likely contributes to the α-cell mass expansion. Unlike β-cells, the expansion of α-cell mass occurs without an accompanying increase in maternal blood glucagon concentrations, except for a transitory increase at E6.5. Some human and rat studies have reported increased maternal blood glucagon concentrations at a time point during pregnancy (26–29). However, conflicting data do not consistently demonstrate increased maternal glucagon production during pregnancy (26–29,32–34). Differences in methodology, animal models, and the timing of glucagon measurements during pregnancy may account for these inconsistencies. Regardless of the maternal blood glucagon concentration, the findings of our study and published data indicate that pregnancy increases α-cell proliferation and α-cell mass (34,61). Most importantly, our study reveals that pregnancy increases maternal pancreatic GLP-1 and glucagon contents, which promote insulin production. Therefore, these data support the concept that maternal pancreatic α-cells adapt to pregnancy at both structural and functional levels.

Glucagon is the primary hormone from α-cells and plays a crucial role in maintaining systemic glucose homeostasis. However, despite ∼96% α-cell ablation, the nonpregnant *α-null* mice in our studies exhibited only minor changes in their metabolic phenotypes. Our results support the notion that despite a robust reduction in α-cell mass, the remaining glucagon in the circulation is sufficient for normal glucose metabolism in *α-null* mice in most physiological conditions (21,48,49). The compensatory effects from other hormone(s) or organs, such as the central nervous system, might also underlie the minor metabolic phenotype of nonpregnant *α-null* mice. In contrast to the improved GTT of nonpregnant *α-null* mice and hypoglycemia of *Gcgr* gene knockout

dams (21,30,31,48,49), significantly increased fed-state serum glucose concentrations and glucose intolerance were observed in *α-null* dams. Furthermore, blocking GCGR signaling showed no significant effect on maternal glucose metabolism. Therefore, these results indicate that the endocrine effects of glucagon play a minor role in regulating maternal glucose metabolism during normal pregnancy. Thus, maternal hyperglycemia produced by α-cell ablation during pregnancy might be caused by a mechanism(s) other than the endocrine effect of glucagon.

The proximity of α- and β-cells in pancreatic islets produces a paracrine effect between these two types of cells (25,62,63). Thus, β-cells normally suppress glucagon secretion from α-cells, but glucagon stimulates glucose-induced insulin secretion (16,64). Importantly, α-cells also secret GLP-1, which enhances glucose-induced insulin secretion (20,65,66). Glucagon and GLP-1 are encoded by the same preproglucagon gene. Posttranslational cleavage of proglucagon by proprotein convertase subtilisin/kexin type 2 (PCSK2) produces glucagon, while PCSK1 directs GLP-1 production (22,62,65,67). Despite the debate about the physiological importance of α-cell–released GLP-1 in glucose metabolism (20), accumulating data indicate that intraislet GLP-1 plays a vital role in augmenting insulin production in conditions of metabolic stresses (19,21,49,51,63,65,68–70). In our study, the pregnancy-induced increase of maternal serum insulin concentration was significantly diminished in *α-null* dams. Similarly, genetic blocking of glucagon and GLP-1 production significantly increases maternal serum glucose concentrations, which is associated with a blunted increase of maternal serum insulin concentrations during pregnancy (71). Most importantly, our study showed that a GLP-1 receptor agonist and GLP-1 itself restored insulin secretion in *α-null* dams and cultured islets from *α-null* dams. Because serum GLP-1 and L-cells were restored in the *α-null* dams, our studies indicate that intraislet GLP-1 plays a specific and vital role in mediating pregnancy-enhanced insulin production. Unlike in the study with systemic *Glp-1r* gene knockout mice (32), both *α-null* mice and the mice with proglucagon gene mutation exhibited normal β-cell proliferation and islet mass expansion during pregnancy (71). In addition, although α- to β-cell transdifferentiation has been proposed as a mechanism for β-cell mass expansion during pregnancy, consistent with two recent studies (33,34), our results do not support this mechanism. Therefore, our combined results lead us to propose that α-cells actively contribute to pregnancy-enhanced insulin production via intraislet GLP-1–promoted insulin secretion. Of note, our data do not rule out the contribution of intraislet glucagon in maternal islet adaptation, and further studies are warranted to test for this possibility. The involvement and regulatory effects of circulating GLP-1 on maternal metabolic adaptation also need to be further studied.

Although pregnancy robustly increases insulin production and maternal blood insulin concentrations in both humans and rodents, there are differences in mechanisms for such β-cell adaptations. Compared with rodents, human β-cells have relatively low rates of proliferation and β-cell mass expansion during pregnancy (45,72,73). Human islets also have significantly more α-cells (making up 35–40% vs. 15–20% of islet cellular content in humans vs. rodents, respectively) (11–13). Without the mantle structure as in rodent islets, human α-cells randomly mix with β-cells and other cells, favoring the potential for intraislet paracrine interactions between α- and β-cells. Further studies will be necessary to confirm the role of α-cells in insulin production and metabolic adaptation during human pregnancy. Such human studies will have the potential to reveal a new cause of insulin insufficiency in gestational diabetes mellitus, namely, failure of α-cell adaptation and/or intraislet GLP-1 production.

In summary, the current study demonstrates that α-cells are a vital component of maternal pancreatic islet adaptation to pregnancy in mouse models. In mice, pregnancy induces α-cell proliferation and α-cell mass expansion. Pancreatic α-cells promote maternal insulin production during pregnancy from adjacent β-cells mainly via GLP-1R–mediated intraislet paracrine action.

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